VYTÓRIA PISCITELLI CAVALCANTI

MEDICINAL PLANTS, *Bacillus subtilis* BV09 AND PRECISION AGRICULTURE IN THE MANAGEMENT OF *Meloidogyne* spp.

LAVRAS-MG
2022

VYTÓRIA PISCITELLI CAVALCANTI

MEDICINAL PLANTS, *Bacillus subtilis* BV09 AND PRECISION AGRICULTURE IN THE MANAGEMENT OF *Meloidogyne* spp.

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Plantas Medicinais, Aromáticas e Condimentares, para obtenção do título de Doutor.

Profª. Drª. Joyce Dória Rodrigues
Orientadora
Prof. Dr. Flávio Henrique Vasconcelos de Medeiros
Coorientador
Prof. Dr. Filipe Almendagna Rodrigues
Coorientador

LAVRAS-MG
2022

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Cavalcanti, Vytória Piscitelli.
Medicinal plants, *Bacillus subtilis* BV09 and precision agriculture in the management of *Meloidogyne* spp. / Vytória Piscitelli Cavalcanti. - 2022.
151 p. : il.

Orientador(a): Joyce Dória Rodrigues.
Coorientador(a): Flávio Henrique Vasconcelos de Medeiros, Filipe Almendagna Rodrigues.
Tese (doutorado) - Universidade Federal de Lavras, 2022.
Bibliografia.

1. Plantas medicinais aromáticas e condimentares. 2. Controle biológico. 3. Agricultura de precisão. I. Rodrigues, Joyce Dória. II. de Medeiros, Flávio Henrique Vasconcelos. III. Rodrigues, Filipe Almendagna. IV. Título.

VYTÓRIA PISCITELLI CAVALCANTI

PLANTAS MEDICINAIS, *Bacillus subtilis* BV09 E AGRICULTURA DE PRECISÃO NO MANEJO DE *Meloidogyne* spp.

MEDICINAL PLANTS, *Bacillus subtilis* BV09 AND PRECISION AGRICULTURE IN THE MANAGEMENT OF *Meloidogyne* spp.

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Plantas Medicinais, Aromáticas e Condimentares, para obtenção do título de Doutor.

APROVADA em 29 de agosto de 2022.
Dr. Vicente Paulo Campos UFLA
Dr. Júlio Carlos Pereira Da Silva UFSM
Dr. Willian César Terra UFLA
Dr. Flávio Henrique Vasconcelos de Medeiros UFLA
Dr. Joyce Dória Rodrigues UFLA

Profª. Drª. Joyce Dória Rodrigues
Orientadora
Prof. Dr. Flávio Henrique Vasconcelos de Medeiros
Coorientador
Prof. Dr. Filipe Almendagna Rodrigues
Coorientador

LAVRAS-MG
2022

AGRADECIMENTOS

Escrever esses agradecimentos é um grande desafio para mim, pois não sei se conseguirei expressar em palavras a gratidão que sinto por tudo o que vivi durante o doutorado e por todos que estiveram comigo nesta trajetória.

Inicio agradecendo à Universidade Federal de Lavras (UFLA), instituição que me ofereceu essa oportunidade e disponibilizou sua infraestrutura para a realização deste trabalho.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

Agradeço à minha orientadora Profª. Drª. Joyce Dória Rodrigues por acreditar e confiar em mim para a realização deste trabalho. Cresci e aprendi muito nesses anos em que trabalhamos juntas, obrigada por tudo!

Aos meus coorientadores Prof. Dr. Flávio Henrique Vasconcelos Medeiros e Prof. Dr. Filipe Almendagna Rodrigues por me apoiarem desde o início do projeto, me incentivando e orientando nos experimentos.

Aos professores por disporem do seu tempo para sanar minhas dúvidas e me orientar nas questões que foram surgindo durante os experimentos. Em especial gostaria de agradecer aos professores Vicente Paulo Campos (DFP-UFLA), Júlio Carlos P. da Silva (UFSM), Valter C. de Andrade Júnior (DAG-UFLA), Júlio Sílvio de S. Bueno Filho (DEX-UFLA), Adão Felipe dos Santos (DAG-UFLA), Rafael Garrett da Costa (LABMETA, UFRJ), Wilson Magela Gonçalves (DAG-UFLA), Cleiton Lourenço de Oliveira (DAG, UFLA), Heloisa O. dos Santos (DAG-UFLA), Maria Fernanda G. V. Peñaflor (DEN-UFLA), Camila Filgueiras (Cornell University) e Denis Willett (Cornell University).

Ao Dr. Willian César Terra por acompanhar os experimentos e escrita dos artigos, me ajudando a acender novas ideias e enxergar além dos números e palavras.

Ao Grupo Vittia por fornecer o Biobaci® e à Mírian R. de Faria pelo suporte técnico no uso do produto biológico.

Ao Laboratório de Cultura de Tecidos Vegetais (DAG-UFLA), Laboratório de Nematologia (DFP-UFLA), Laboratório de Controle Biológico (DFP-UFLA), Setor de Olericultura (DAG-UFLA) e Centro de Desenvolvimento e Transferência de Tecnologia (CDTT-UFLA), onde realizei os experimentos. À equipe do CDTT, em Ijaci-MG, pelos aprendizados e por me ajudarem durante todo o experimento no campo. Foi um prazer enorme estar com vocês.

Aos estudantes da graduação que me ajudaram na realização dos experimentos. Vocês me ensinaram que apesar de parecer impossível, quando trabalhamos em equipe conseguimos ultrapassar as expectativas. Aprendi muito com vocês! Agradeço em especial aos amigos Clerio, Kamilly, Brenda, Sansão, Regiane, Maria Luiza, Otávio, Natália, Lílian, Ana Luiza, Arthur e à equipe do Grupo de Extensão e Pesquisa em Agricultura Digital (GEPAD).

Aos técnicos que me auxiliaram nas diversas atividades, em especial Vantuil, Celso, Giulia, Giuliana, Stefany, Sílvio, Luísa, Leando (Dico), Paulinho, Anete e Evaldo.

Às secretárias Marli e Ana Luiza por todo auxílio.

Ao Dr. Wilson V. S. Pereira pela sua disponibilidade e ajuda em algumas análises.

Aos funcionários da limpeza na UFLA que sempre estiveram junto comigo no dia a dia do laboratório tornando o nosso ambiente de trabalho mais agradável e divertido, em especial agradeço à Sirlene, Priscila, Everaldo, Carol, João e Luciana.

Aos grupos de estudos que tive o prazer de fazer parte, os quais me proporcionaram muitos aprendizados e crescimento profissional e pessoal: Núcleo de estudos em Plantas

Medicinais, Aromáticas e Condimentares (NEMAC), Núcleo de estudos em Produtos Naturais (NEPRON), Núcleo de estudos em Agroecologia, Permacultura e Extensão Inovadora (NEAPE), Núcleo de estudos em Cultura de Tecidos Vegetais (NECULT) e Núcleo de estudos em Paisagismo e Floricultura (NEPAFLOR).

Aos professores do programa de pós-graduação em Plantas Medicinais, Aromáticas e Condimentates por me ensinarem tanto e por aumentarem ainda mais o meu encanto pela beleza e poder das plantas medicinais, em especial aos professores Suzan Kelly Vilela Bertolucci, José Eduardo Brasil Pereira Pinto, Luciane Vilela Resende e Manuel Losada Gavilanes que me acompanharam desde o mestrado.

Aos professores Andrea Aparecida Ribeiro Correa e Gilmar Tavares pela amizade e pelos ensinamentos.

Ao professor Victor Makins da VMLI® pelas aulas de inglês divertidas e que me ajudaram muito durante o doutorado.

Aos meus pais Ângela e Fernando, meus irmãos Pórtya, Crystal e Arthur, meus avós e familiares por estarem sempre ao meu lado, comemorando minhas conquistas e me amparando nas dificuldades. Meu porto seguro, amo vocês!

Ao meu noivo Túlio Junqueira Carneiro por estar sempre ao meu lado me apoiando e incentivando, me alegrando e ajudando a tornar esse processo mais leve. Te amo!

À Helena Maria, Dimas, Mara e Joana pelo apoio durante esta jornada e por me receberem com tanto amor em sua família, amo vocês. Jô, muito obrigada por me incentivar a fazer a pós-graduação, essa experiência incrível que me abriu muitas portas. Gratidão!

À Raquel Azevedo pela amizade e por cuidar de mim em Lavras, me ajudando a enxergar as infinitas possibilidades que a vida nos oferece todos os dias.

À Nathalia Assis, minha psicóloga e amiga que me acompanhou durante todo o doutorado, me ajudando a superar meus medos e a enfrentar todos os desafios, comemorando comigo cada conquista. Você fez parte do meu crescimento, sinto muito sua falta!!

Aos amigos que me acompanharam durante o doutorado tornando a jornada mais leve e divertida, muito obrigada! Aos amigos do laboratório de cultura de tecidos vegetais, onde sempre tem café e muita prosa boa, em especial agradeço ao Vantuil, Celso, Filipe, Ivan, Altino, Lílian, Ana Luiza, Arthur, Ronilson, Ana Milena, Yulimar, Cristina, Leandro. Aos amigos do laboratório de nematologia por me receberem de braços abertos, me ajudando nos experimentos e me dando apoio emocional nessa reta final. Muito obrigada Daniele, Letícia, Larissa, Paulo e Willian. Aos amigos do programa de plantas medicinais agradeço em especial à Sâmia, Diene, Raíssa, Marlon, Thainá, Júlia, Janina, João Pedro, Adriane, Maria de Fátima e Jeimmy. Agradeço ao Jeferson da batata-doce por ser essa pessoa tão especial, sempre disposto a ajudar e contagiando todos com sua felicidade e boas energias.

Às minhas irmãs de república Kize, Cristiane, Maria Jéssica, Fernanda, Louise, Mara, Daiane e Jaqueline. Amo vocês, contem sempre comigo!

À Andrea e à toda Colônia Esperança por me acolherem com tanto carinho e por serem luz na vida de tantas pessoas.

Àqueles que me ajudaram e não encontraram o nome citado aqui nos meus agradecimentos, por favor, me perdoem e tenham certeza que sou grata por tudo que fizeram por mim e vocês estarão em minhas orações. Espero que entendam que a correria está tão grande para terminar de escrever a tese e minha cabeça está tão atordoada que eu provavelmente vou acabar me esquecendo de mencionar pessoas importantes.

Enfim, finalizo o doutorado sabendo que me esforcei e fiz sempre o melhor que eu pude em cada situação. Aprendi muito nessa trajetória acadêmica e espero estar conseguindo retribuir à sociedade por apoiar o ensino gratuito neste país.

RESUMO

Alface e tomate são hortaliças de alto valor comercial amplamente consumidas no mundo. Os nematoides-das-galhas (*Meloidogyne* spp.) são fitoparasitas que provocam a formação de galhas no sistema radicular de ambas afetando seu crescimento, podendo causar perdas em mais de 80% da produção nestas culturas. Para controlar fitonematoides é necessário fazer o manejo integrado, adotando práticas de controle cultural, químico e biológico. Algumas plantas medicinais podem ser utilizadas no controle de *Meloidogyne* spp. devido à sua capacidade de produzir metabólitos com propriedades nematicidas. Nesse contexto, objetivou-se avaliar o efeito do cultivo de plantas medicinais em consórcio com alface e tomateiro no manejo de nematoide-das-galhas nestas culturas. Considerando a importância do manejo integrado no controle de fitonematoides e a busca por alternativas menos agressivas ao ambiente, em experimento no campo foram feitas aplicações de *Bacillus subtilis* BV09 (nematicida microbiológico), eliminação das raízes infestadas, pousio. Imagens aéreas capturadas por veículos aéreos não tripulados (VANT) podem fornecer informações importantes para a tomada de decisão nos tratos culturais, detecção e manejo da doença, portanto, foram capturadas imagens aéreas para monitoramento da lavoura no campo. Inicialmente, foram selecionadas três espécies medicinais: *Allium sativum* (alho), *Catharanthus roseus* (vinca) e *Achillea millefolium* (mil-folhas). *Tagetes patula* (cravo-de-defunto) foi utilizada como controle positivo. Estas foram cultivadas em consórcio com alface ou tomateiro em vasos (experimentos 1 e 2). O substrato foi infestado com ovos ou juvenis de segundo estádio (J2) de *Meloidogyne javanica* e os vasos foram mantidos em casa de vegetação até a avaliação do número de ovos nas raízes. As plantas medicinais em consórcio com alface ou tomateiro promoveram redução na população total de *M. javanica*. Em laboratório verificou-se atratividade das raízes e exsudatos radiculares, penetração reduzida e J2 com desenvolvimento afetado nas raízes de vinca e cravo-de-defunto, enquanto os J2 apresentaram resposta aleatória aos exsudatos radiculares de mil-folhas e alho. No campo (experimento 3) foi avaliado o efeito do cultivo de alho e aplicação de *Bacillus subtilis* BV09 no manejo de *Meloidogyne incognita*, além do uso de imagens aéreas para monitoramento da doença. Cultivo de alho em consórcio com alface, aplicação de *B. subtilis* BV09, assim como solo em pousio e eliminação das raízes infestadas, foram eficientes na redução da população de *M. incognita* no campo. A produção por planta de alho foi maior no consórcio com alface e aplicação de *B. subtilis* BV09, enquanto a produção por planta de alface foi afetada no consórcio quando as plantas de alho estavam grandes, competindo por nutrientes e luz. O consórcio possibilitou a obtenção de elevada renda total das culturas de alface e alho, assim como a renda total obtida com o monocultivo de alface. Em laboratório observou-se que a aplicação de *B. subtilis* BV09 alterou a composição química dos exsudatos radiculares de alface e alho afetando a resposta quimiotática de *M. incognita*. As imagens aéreas obtidas com VANT permitiram a avaliação do crescimento das plantas de alface ao longo do tempo, apresentando potencial para uso na detecção de áreas afetadas por nematoide-das-galhas quando as plantas apresentam crescimento reduzido.

Palavras-chave: Nematoide-das-galhas. Controle cultural. Controle biológico. Imagens aéreas.

ABSTRACT

Lettuce and tomato are vegetables of high commercial value widely consumed around the world. Root-knot nematodes (*Meloidogyne* spp.) are soil-borne pathogens that causes galls formation in the root system of both affecting plants' growth, which can cause losses in more than 80% of production in lettuce and tomato crops. To control plant-parasitic nematodes, integrated management is necessary, adopting cultural, chemical and biological control practices. Some medicinal plants can be used to control *Meloidogyne* spp. due to its ability to produce metabolites with nematicidal properties. In this context, the objective was to evaluate the effect of cultivating medicinal plants intercropped with lettuce and tomato on the management of root-knot nematode in these crops. Considering the importance of the integrated management in nematodes control and the search for alternatives less aggressive to the environment, in the field experiment applications of *Bacillus subtilis* BV09 (microbiological nematicide), elimination of infested roots, and fallow. Aerial images captured by unmanned aerial vehicles (UAV) can provide important information for decision-making in cultural treatments, detection and management of plant diseases, therefore, aerial images were carried out to monitor the crops. Initially, three medicinal species were selected: *Allium sativum* (garlic), *Catharanthus roseus* (Madagascar periwinkle), and *Achillea millefolium* (yarrow). *Tagetes patula* (marigold) was used as a positive control. These were grown intercropped with lettuce and tomato in pot (experiments 1 and 2). The substrate was infested with eggs or second-stage juveniles (J2) of *Meloidogyne javanica* and the pots were kept in a greenhouse until the evaluation of the number of eggs in plant roots. Medicinal plants intercropped with lettuce or tomato promoted a reduction in the total population of *M. javanica* per pot. In laboratory, it was found attractiveness of roots and root exudates, reduced penetration and affected the development of J2 in the roots of Madagascar periwinkle and marigold, while J2 showed a random response to yarrow and garlic root exudates. In the field (experiment 3) the effect of garlic cultivation and the application of *B. subtilis* BV09 on the management of *Meloidogyne incognita* was evaluated, as well as the use of aerial images to monitor the disease. Garlic cultivation intercropped with lettuce, application of *B. subtilis* BV09, fallow soil and elimination of infested roots were efficient in reducing *M. incognita* population in the field. The production per garlic plant was improved by intercropping with lettuce and *B. subtilis* BV09 application, while the production per lettuce plant was affected by the intercropping when garlic plants were large, due to competition for nutrients and solar radiation. Intercropping enabled achieving high total income from lettuce and garlic crops, as the total income obtained from lettuce monoculture. In laboratory, it was observed that *B. subtilis* BV09 application altered the chemical composition of lettuce and garlic root exudates, affecting the chemotactic response of *M. incognita*. Aerial images obtained with UAV allowed the evaluation of lettuce plants' growth over time, presenting the potential for use in the detection of root-knot nematode affected areas when plants show reduced growth.

Keywords: Root-knot nematode. Cultural control. Biological control. Aerial images.

SUMÁRIO

1 INTRODUÇÃO

Nos últimos tempos os produtores vêm enfrentando problemas com pragas e doenças no campo, provocando redução na produção e aumento no investimento em estratégias de controle visando evitar perdas econômicas. Os nematoides parasitas de plantas têm causado elevado nível de danos às culturas (RALMI; KHANDAKER; MAT, 2016; SINGH; SINGH; SINGH, 2015). As espécies do gênero *Meloidogyne*, conhecidas como nematoide-das-galhas, apresentam ampla gama de hospedeiros e ampla distribuição mundial, sendo consideradas economicamente importantes para diversas culturas (SIKORA; FERNANDEZ, 2005). Entre os hospedeiros estão várias hortaliças como o alface (*Lactuca sativa* L.) e o tomateiro (*Solanum lycopersicum* L.), importantes no cenário mundial e cujas perdas causadas por *Meloidogyne* spp. podem atingir mais de 80% de produção (CHARCHAR; MOITA, 2005; PINHEIRO; PEREIRA; SUINAGA, 2014; SEID et al., 2015).

O controle de fitonematoides é oneroso e a sua erradicação é praticamente impossível, tornando-se necessária a implantação de um conjunto de medidas para obter sucesso no controle, como a utilização de mudas sadias, plantio de cultivares resistentes, eliminação das plantas hospedeiras dos nematoides, manipulação de áreas em alqueive, controle químico, controle biológico, uso de plantas não hospedeiras na rotação de culturas e consórcio com espécies que apresentam efeito antagônico aos fitonematoides (PINHEIRO; PEREIRA; SUINAGA, 2014; RALMI; KHANDAKER; MAT, 2016).

As plantas medicinais, além de sua importância na saúde humana e animal, são amplamente estudadas no controle de pragas e doenças, sendo capazes de produzir metabólitos secundários com atividade nematicida (D'ADDABBO et al., 2017; MATEUS et al., 2014), assim como compostos orgânicos voláteis capazes de manipular a migração dos nematoides (ALI; ALBORN; STELINSKI, 2011; FILGUEIRAS et al., 2016; TORTO et al., 2018a). Paralelamente, através do cultivo de plantas medicinais os agricultores podem obter renda maior do que cultivando outras culturas comerciais e tradicionais (SINGH et al., 2019).

Outra estratégia para o manejo de nematoides parasitas de plantas que está crescendo nos últimos anos é a aplicação de produtos biológicos, por ser uma prática sustentável que apresenta resultados satisfatórios além de seu menor impacto ao ambiente. No Brasil existem cerca de 47 nematicidas microbiológicos disponíveis no mercado, sendo encontradas 11 diferentes espécies de microrganismos como ingrediente ativo, havendo predominância de produtos a base de bactérias do gênero *Bacillus* (AGROFIT, 2022; MACHADO, 2022).

O ataque de nematoide-das-galhas (*Meloidogyne* spp.) provoca a formação de galhas no sistema radicular da planta, diminuindo a absorção de água e nutrientes do solo e provocando deficiências minerais. Redução no vigor das plantas, sintomas de deficiência nutricional e perdas na produtividade são observados no campo na forma de reboleiras de formato irregular (PINHEIRO; PEREIRA; SUINAGA, 2014; SEID et al., 2015; SILVA, 2015). Nesse cenário, buscando simplificar o processo de detecção e monitoramento da ocorrência da doença e reduzir as perdas, imagens obtidas de veículos aéreos não tripulados (VANT) podem ser utilizadas para obter dados do crescimento e estado nutricional das plantas em campos infestados com *Meloidogyne* spp., fornecendo informações valiosas para a tomada de decisões pelos agricultores nos tratos culturais e no manejo da doença (BARBEDO, 2019; RAPARELLI; BAJOCCO, 2019).

Neste contexto, o objetivo do trabalho foi avaliar o efeito do cultivo de plantas medicinais antagônicas a fitonematoides no manejo de *Meloidogyne* spp. nas culturas de alface e tomateiro. Adicionalmente, considerando a importância de se utilizar mais de uma estratégia no manejo de fitonematoides, no experimento de campo também foram avaliados o efeito da aplicação de *Bacillus subtilis* BV09 e o uso da agricultura de precisão no manejo de *M. incognita*.

2 REFERENCIAL TEÓRICO

2.1 Cultura da alface e do tomateiro

A alface (*Lactuca sativa* L., Asteraceae) é uma hortaliça folhosa consumida em todo o mundo. Em 2017, movimentou 1,2 bilhões de reais com produção de 671.509 toneladas (IBGE, 2022a). É uma espécie anual de ciclo curto, em torno de 30 dias no verão e 45 dias no inverno, permitindo a realização de vários ciclos durante o ano e com rápido retorno financeiro (MALDONADE; MATTOS; MORETTI, 2014; YOKORO; PEREIRA, 2020).

O tomateiro (*Solanum lycopersicum* L.) é uma hortaliça amplamente cultivada no mundo. No Brasil em 2020 o valor da produção de tomate chegou a 6,04 bilhões de reais, com produção de 3,75 milhões de toneladas em 51,96 mil hectares de área colhida e rendimento médio de 72,24 mil quilos por hectare (IBGE, 2022b). Trata-se de uma cultura anual cuja colheita pode ser realizada entre 90 e 120 dias depois da semeadura (NAIKA et al., 2006). O tomate é um fruto bastante valorizado por apresentar compostos bioativos antioxidantes, como licopeno, compostos fenólicos e carotenoides (TAMASI et al., 2019).

Ao longo do tempo a intensificação agrícola vem provocando desequilíbrio ambiental, aumentando a população de pragas e patógenos até atingirem níveis em que causam perdas de produtividade e, consequentemente, danos econômicos (BARRIÈRE et al., 2014). Entre as pragas e doenças que acometem essas culturas destacam-se os nematoides-das-galhas (*Meloidogyne* spp.) (ELLING, 2013), que são capazes de provocar perdas maiores do que 80% na produção de alface (CHARCHAR; MOITA, 2005) e tomate (EMBRAPA, 1993; PINHEIRO; PEREIRA; SUINAGA, 2014; SEID et al., 2015).

Quando as plantas são atacadas de maneira severa por *Meloidogyne* spp. ocorre elevada formação de galhas, o sistema radicular fica desorganizado e com poucas raízes, além da planta ficar com aspecto clorótico e haver diminuição no crescimento, podendo reduzir o número e a qualidade dos frutos. A infestação pode ocorrer desde o estádio de plântula, podendo no transplante para o campo ocorrer a morte das plântulas, ou estas podem sobreviver sofrendo danos em seu crescimento e produtividade (SILVA, 2015; VALE; LOPES; ALVARENGA, 2004).

2.2 Nematoide-das-galhas (*Meloidogyne* spp.)

Os nematoides fitoparasitas costumam ser negligenciados e dificilmente controlados devido à dificuldade de sua visualização, uma vez que ocorrem principalmente abaixo do solo, e pelo fato dos seus sintomas na planta não serem específicos. Causam danos diretos e indiretos nas plantas, deixando-as predispostas ao ataque de outros patógenos, podendo formar complexos de doenças e aumentar os danos às culturas (SILVA, 2015; SINGH; SINGH; SINGH, 2015). Os fitonematoides causam perdas em hospedeiras comerciais em todo o mundo, sendo observadas perdas entre 25 e 100% no rendimento de culturas, com prejuízo anual estimado de cerca de US$ 157 bilhões (SEID et al., 2015; SINGH; SINGH; SINGH, 2015).

A espécie *M. incognita* é amplamente distribuída pelo mundo e, apesar de ser parasita obrigatória de raízes e caules subterrâneos, apresenta ampla variedade de plantas hospedeiras, e isso favorece a sua permanência no campo (PINHEIRO; PEREIRA; SUINAGA, 2014; SEID et al., 2015). Por ser uma espécie cosmopolita e generalista bem adaptada às condições climáticas das regiões tropicais e subtropicais, torna-se importante economicamente para diversas culturas (SIKORA; FERNANDEZ, 2005).

A duração do ciclo de vida dos nematoides-das-galhas ocorre em torno de 3 a 5 semanas e cada fêmea produz em média 500 ovos, os quais ficam envolvidos por uma

substância gelatinosa formando uma massa de ovos que os mantêm protegidos contra condições adversas, como a dessecação (OLIVEIRA; ROSA, 2014; PINHEIRO; PEREIRA; SUINAGA, 2014; SILVA, 2015). A exposição dos ovos às condições ambientais favoráveis, como temperaturas por volta de 28ºC e umidade na capacidade de campo, provoca o desenvolvimento embrionário e a eclosão dos juvenis de segundo estádio (J2) em aproximadamente nove dias (CAMPOS; CAMPOS; POZZA, 2008; PINHEIRO; PEREIRA; SUINAGA, 2014). Os J2, que representam a forma infectiva, assim que eclodem podem diretamente infectar outra raiz ou vão para o solo, provocando aumento no nível de inoculo e, consequentemente, aumentando os danos à cultura (KANKAM; ADOMAKO, 2014; PINHEIRO; PEREIRA; SUINAGA, 2014).

O ataque de *M. incognita* pode ocorrer durante todo o ano e provoca a formação de galhas, diminuindo a absorção de água e nutrientes do solo, levando a deficiências minerais, redução no volume do sistema radicular, murchamento das plantas, redução do vigor, perdas na produtividade e redução na qualidade do produto final, sendo estes sintomas encontrados no campo na forma de reboleiras de formato irregular (PINHEIRO; PEREIRA; SUINAGA, 2014; SEID et al., 2015; SILVA, 2015).

2.3 Estratégias para o manejo de *Meloidogyne* spp.

Em áreas infestadas com fitonematoides a total eliminação destes é impossível e economicamente inviável, sendo importante aprender a conviver com eles no campo, realizando monitoramentos periódicos e a utilização de métodos capazes de reduzir a população deste patógeno a níveis abaixo do limiar de dano econômico (EMBRAPA, 1993; SILVA, 2015).

Entre os métodos aplicados no manejo de nematoides parasitas de plantas é importante destacar medidas de prevenção, como quarentena e sanidade das mudas, principalmente em regiões que não apresentam problemas com estes patógenos. Enquanto em regiões já infestadas, são importantes medidas de controle: (1) tratamentos físicos do solo, entre eles a solarização ou aplicação de calor de vapor e inundação, (2) rotação de culturas com espécies que não são hospedeiras, (3) biofumigação, (4) controle químico, (5) controle biológico, (6) utilização de sistemas de cultivo sem solo (exemplo: hidroponia), (7) cultivares resistentes e (8) manejo integrado de nematoides, que visa melhorar o rendimento da cultura usando uma combinação de opções de manejo, tendo como alvo o controle de espécies-chave de

nematoides e levando em consideração o equilíbrio do ecossistema dentro das medidas adotadas (SEID et al., 2015; SUBEDI; THAPA; SHRESTHA, 2020).

2.4 Plantas no manejo de *Meloidogyne* spp.

As plantas antagônicas a nematoides fitopatogênicos e seus constituintes químicos têm potencial para desempenhar um importante papel no manejo sustentável dos fitonematoides. O cultivo dessas plantas é uma ferramenta fundamental em sistemas orgânicos, considerando a pequena quantidade de métodos de controle admitidos. As plantas antagônicas e seus constituintes químicos também podem ser aplicados na agricultura convencional em culturas de ciclo curto, visando substituir os nematicidas sintéticos e evitando o risco de resíduos no produto final. Além disso, a combinação de formulações de plantas com nematicidas sintéticos pode ser recomendada para culturas de ciclo longo e/ou na presença de altas densidades iniciais de nematoides parasitas de plantas como estratégia complementar no manejo do fitonematoide (D'ADDABBO et al., 2014).

2.4.1 Consórcio, rotação e sucessão de culturas

Em áreas infestadas com nematoide-das-galhas, as espécies cultivadas e a forma como estas são distribuídas no campo devem ser consideradas ao desenvolver estratégias de manejo. Ao considerar que o nematoide-das-galhas (*Meloidogyne* spp.) é um parasita obrigatório de plantas que invade as raízes para seu crescimento, desenvolvimento e reprodução (PINHEIRO; PEREIRA; SUINAGA, 2014; SEID et al., 2015), ao realizar o consórcio, rotação ou sucessão de culturas com espécies não hospedeiras ocorre a redução na população de nematoide-das-galhas no solo ao promover redução na quantidade de raízes que este nematoide é capaz de parasitar e, consequentemente, redução no seu potencial de reprodução (ABD-ELGAWAD, 2021). Além disso, as plantas não hospedeiras podem liberar no solo exsudatos radiculares contendo substâncias tóxicas que atuam de diversas maneiras no controle de nematoide-das-galhas, suprimindo a eclosão de J2s, afetando o desenvolvimento dos juvenis, atingindo o sistema nervoso dos juvenis e bloqueando a atração dos juvenis que estão no solo procurando por um hospedeiro (TORTO et al., 2018b).

Rotação e sucessão de culturas são técnicas agrícolas diferentes, sendo a rotação de culturas caracterizada pela alternância anual de espécies vegetais no mesmo local e na mesma estação de cultivo em anos consecutivos, enquanto a sucessão de culturas é caracterizada pelo

cultivo de duas ou mais espécies de plantas em sequência na mesma área de cultivo durante o período de até um ano (LOURENÇO JR.; LOPES; REIS, 2016). Tanto a rotação quanto a sucessão de culturas podem ser aplicadas como estratégias para o manejo populacional de fitonematoides com resultados satisfatórios quando são utilizados cultivares resistentes e culturas não hospedeiras e supressoras de nematoides fitopatogênicos (KRATOCHVIL et al., 2004; SILVA et al., 2022; TORTO et al., 2018b). No manejo de nematoides do gênero *Meloidogyne* spp. a rotação ou sucessão de culturas torna-se um desafio considerando a elevada quantidade de plantas hospedeiras, deixando de ser uma estratégia de manejo em sistemas de cultivo que utilizam apenas culturas suscetíveis (KRATOCHVIL et al., 2004).

O consórcio de culturas é caracterizado pelo cultivo simultâneo de mais de uma espécie vegetal na mesma área agrícola (SANTOS et al., 2007). O cultivo de plantas em consórcio é uma estratégia capaz de atrair inimigos naturais, aumentar a biodiversidade reduzindo o recurso disponível para as pragas e doenças se estabelecerem, manipular as condições do solo na área de plantio e induzir a supressividade do solo a patógenos (COOK; KHAN; PICKETT, 2007; PICKETT et al., 2014; RATNADASS et al., 2012; SILVA; MEDEIROS; CAMPOS, 2018). No manejo de nematoide-das-galhas, o cultivo em consórcio pode ser feito intercalando a cultura principal com espécies não hospedeiras, sendo necessário o uso de plantas não hospedeiras com ciclo mais longo do que a cultura principal (TORTO et al., 2018b). Em sistemas de cultivo consorciado podem ser enfrentados problemas como a competição das plantas companheiras com a cultura principal. Essa competição pode provocar efeito negativo sobre o rendimento da cultura, sendo importante estudar a densidade de plantas para evitar a competição, assim como evitar plantas que apresentem efeito alelopático desfavorável (TRINGOVSKA et al., 2015).

Diversos fatores devem ser considerados quando se deseja realizar estas estratégias de manejo no campo, desde os seus efeitos positivos e negativos para a cultura principal, até os efeitos sobre a comunidade que habita esse determinado ambiente. Dessa forma, é importante que as estratégias de manejo a serem aplicadas não provoquem perdas de grupos funcionais presentes no ecossistema, como a população de microrganismos no solo que apresenta importantes agentes de controle biológico, assim como os insetos que podem ser inimigos naturais de pragas, entre outros. O uso de culturas complementares com efeito supressivo a nematoides trata-se de um componente com potencial para aplicação em manejos integrados e produção orgânica, sendo essencial o equilíbrio entre os organismos dentro do ambiente de produção para a estabilidade e o funcionamento do ecossistema (KEBEDE et al., 2018; LUPATINI et al., 2019; TRINGOVSKA et al., 2015).

Lupatini et al. (2019) observaram aumento na diversidade da comunidade fúngica e na população de nematoides de vida livre em sistema agrícola orgânico, quando comparado ao sistema agrícola convencional, além de ter suprimido os nematoides parasitas de plantas pertencentes às famílias Meloidogynidae e Pratylenchidae. Nesse sentido, visando a aplicação de métodos de controle de fitopatógenos mais sustentáveis, é essencial o conhecimento dos efeitos desses sistemas de cultivo na modulação da comunidade do solo e na produtividade das plantas (LUPATINI et al., 2019).

2.4.2 Resposta dos nematoides a estímulos químicos

A movimentação dos nematoides no solo ocorre em uma matriz tridimensional e estes respondem a uma variedade de estímulos, que podem ser químicos e/ou físicos (DUSENBERY, 1989; PERRY, 1996). O sistema nervoso do nematoide é composto por órgãos sensoriais e funciona através da relação entre o estímulo, a recepção e os comportamentos resultantes. Os nematoides orientam-se quimicamente pela comparação simultânea da intensidade dos estímulos ao longo do comprimento do corpo nos órgãos quimiossensores, destacando-se entre estes os dois anfídios na região labial e dois fasmídeos na cauda do nematoide, os quais estão envolvidos na identificação de plantas hospedeiras (CURTIS, 2008).

O comportamento dos nematoides na procura de hospedeiros pode ser manipulado tanto por sinais do(s) hospedeiro(s), quanto por fatores do ambiente ao redor, como umidade, temperatura, potencial elétrico, dióxido de carbono, pH e vários compostos inorgânicos e orgânicos (ALI; ALBORN; STELINSKI, 2011; CURTIS, 2008; DUSENBERY, 1989; WANG; BRUENING; WILLIAMSON, 2009). Hida et al. (2015) mostraram que o nitrato de potássio (KNO_3) pode ter um efeito repelente ou atrativo para *M. incognita*, dependendo da sua concentração. Os estímulos químicos no ambiente podem decorrer de compostos voláteis, que se difundem pelo solo, ou compostos solubilizados no filme de água que se movimenta entre as partículas do solo (ALI; ALBORN; STELINSKI, 2011).

2.4.3 Exsudatos de plantas

Os exsudados radiculares são definidos como substâncias de baixo peso molecular que são liberadas pelas raízes das plantas no meio circundante, como açúcares, aminoácidos,

peptídeos, enzimas, vitaminas, ácidos orgânicos, nucleotídeos, fenólicos e outros metabólitos secundários (HAICHAR et al., 2014; ROVIRA, 1969). Estes desempenham importantes papéis na ecologia subterrânea, podendo apresentar atividades biológicas específicas, porém, como a exsudação geralmente ocorre em pequenas quantidades, tão baixas que tais compostos são dificilmente detectáveis por técnicas químicas ou cromatográficas, os bioensaios são amplamente utilizados para sua avaliação (HAICHAR et al., 2014; RASMANN et al., 2012; ROVIRA, 1969).

Os metabólitos secundários presentes nos exsudatos radiculares liberados no solo são importantes na mediação da interação da planta com pragas e patógenos, como insetos herbívoros e nematoides parasitas de plantas, assim como na mediação de interações entre plantas no ambiente. Essa complexa mistura de substâncias liberadas pelas raízes abaixo do solo é caracterizada por compostos pertencentes a várias famílias químicas, principalmente terpenoides, bem como derivados de ácidos graxos, ou compostos contendo enxofre (DELORY et al., 2016).

Entre os metabólitos secundários estão os compostos orgânicos voláteis (COVs), que são geralmente líquidos lipofílicos de alta pressão de vapor, capazes de atravessar membranas celulares e sendo facilmente liberados na atmosfera ou no solo, quando não existem barreiras de difusão (PICHERSKY et al., 2006). Após sua liberação, a transmissão de COVs através do solo é relativamente fácil, como é o caso de raízes de plantas que, quando danificadas por larvas de lagarta, podem liberar compostos voláteis com ampla mobilidade no solo que atraem nematoides entomopatogênicos, sendo apontado que a sua liberação pelas raízes está relacionada com a exposição da planta a estresses bióticos e/ou abióticos (DELORY et al., 2016; PICKETT; KHAN, 2016).

A produção dos exsudatos varia entre as diferentes espécies de plantas, assim como diversos fatores podem influenciar as taxas de exsudação, como os genótipos dentro de uma mesma espécie, a idade e o estágio de desenvolvimento da planta. Enquanto as plântulas geralmente produzem pequenas quantidades de exsudatos radiculares, durante o seu desenvolvimento ocorre um aumento gradual da produção de exsudatos até o florescimento, a qual começa a decair à medida que a planta atinge a maturidade (HAICHAR et al., 2014).

Diversas plantas produzem “células da borda” na raiz, tratando-se de células metabolicamente ativas que se separam e são liberadas da coifa da raiz para o ambiente externo, cujos exsudatos podem atrair e imobilizar nematoides, entre outras diversas funções, desempenhando papel fundamental na proteção da ponta da raiz (HAICHAR et al., 2014; ROCHA; CAMPOS, 2004).

Pesquisas sobre a ecologia subterrânea destacam as diversas interações que ocorrem na rizosfera mediadas por fotoassimilados que são excretados pelas raízes das plantas, cuja composição é responsável pelos estímulos contraditórios, simultaneamente atraente e repulsivo, em relação aos microrganismos do solo (HAICHAR et al., 2014; RASMANN et al., 2012; TORTO et al., 2018a). Os exsudatos radiculares apresentam numerosas funções no controle de processos abióticos e bióticos, como a alteração das propriedades químicas e físicas do solo, inibindo o crescimento de plantas competidoras, combatendo herbívoros e regulando a comunidade microbiana (HAICHAR et al., 2014; RASMANN et al., 2012; SOM; WILLETT; ALBORN, 2017). As raízes liberam exsudatos com substâncias químicas que podem atrair o estágio infeccioso dos nematoides parasitas de plantas e recentemente houve progresso significativo na identificação dessas moléculas sinalizadoras (TORTO et al., 2018a).

Ali, Alborn e Stelinski (2011) observaram em seus resultados que nas raízes de espécies de citros podem ser liberados sinais atrativos para nematoides, atuando sobre diferentes espécies de nematoides, tanto entomopatogênicos, quanto parasitas de plantas. A liberação dos sinais atrativos ocorreu tanto de forma constitutiva, encontrada apenas em uma cultivar resistente, bem como induzida por herbivoria, observada em linhagens suscetíveis a espécies de nematoides patogênicos. O contrário também pode acontecer, como é o caso dos exsudatos radiculares da mamona (*Ricinus communis* L.) que contêm ácidos graxos, ácido palmítico e ácido linoleico que repelem *M. incognita* (DONG et al., 2018).

Além da presença de moléculas sinalizadoras nos exsudatos radiculares, estes também podem apresentar moléculas antagonistas aos fitonematoides, ou seja, que apresentam efeitos negativos ao nematoide ou que são nematicidas. São conhecidos diversos compostos bioativos de plantas com essa ação, como politienilos, isotiocianatos, glicosinolatos, glicosídeos cianogênicos, poliacetilenos, alcaloides, lipídios, terpenoides, sesquiterpenoides, diterpenoides, triterpenos limonoides, quassinoides, esteroides, triterpenoides, compostos fenólicos simples e complexos, flavonoides, saponinas, taninos, óleos essenciais, ácidos graxos, entre outros (CHITWOOD, 2002; D'ADDABBO et al., 2014; DONG et al., 2018).

2.5 Plantas medicinais no manejo de *Meloidogyne* spp.

Vem crescendo o interesse pelo uso de plantas medicinais na manutenção da saúde, tanto para prevenir, quanto para tratar doenças, visto que as plantas medicinais são amplamente utilizadas devido à sua eficiência (cientificamente comprovada para diversas

espécies), ao seu baixo custo, atendendo diferentes classes sociais, e pela facilidade na utilização, sendo um hábito tradicional passado de geração para geração, tratando-se de um mercado promissor (JAMSHIDI-KIA; LORIGOOINI; AMINI-KHOEI, 2018; KLEIN et al., 2009). No Brasil, entre os anos de 2013 e 2015 o Sistema Único de Saúde (SUS) teve um aumento de 161% na procura por tratamentos naturais com plantas medicinais e medicamentos fitoterápicos (MINISTÉRIO DA SAÚDE, 2016). As propriedades farmacológicas das plantas medicinais têm sido consideradas promissoras para o desenvolvimento de novos medicamentos para os cuidados com a saúde humana e animal e, consequentemente, estão crescendo os esforços no desenvolvimento de novos medicamentos a partir de plantas (DUTRA et al., 2016; SINGH, 2015). O mercado brasileiro de plantas medicinais foi estimado em cerca de US$ 400 milhões ao ano, com crescimento anual em torno de 12% (CARVALHO et al., 2018), enquanto o mercado global foi estimado em cerca de US$ 62 bilhões por ano, com crescimento anual em torno de 10 a 20% (ALAMGIR, 2017).

Além do importante papel das plantas medicinais na saúde humana e animal, elas também são eficientes no manejo de pragas e doenças de plantas, tratando-se de uma alternativa segura em comparação ao uso de agrotóxicos (STEVENSON; ISMAN; BELMAIN, 2017; ZAKER, 2016). Elas são capazes de produzir metabólitos secundários com atividade nematicida (D'ADDABBO et al., 2017; MATEUS et al., 2014), assim como compostos orgânicos voláteis capazes de manipular a migração dos nematoides (ALI; ALBORN; STELINSKI, 2011; FILGUEIRAS et al., 2016; TORTO et al., 2018a). Além da aplicação na forma de produtos biocidas, as plantas medicinais também podem ser utilizadas no controle de patógenos no campo através de técnicas agronômicas, como adubos verdes, rotação de culturas ou plantio da cultura principal em consórcio com plantas antagônicas aos fitopatógenos (D'ADDABBO et al., 2014; DONG et al., 2018; ISMAIL, 2013; VESTERGÅRD, 2019).

O uso das plantas medicinais no manejo de fitonematoides pode trazer outros benefícios, como melhorar parâmetros de crescimento do tomateiro e aumentar a sua produtividade (MASHELA et al., 2007), ou pode acarretar em desvantagens, como provocar efeitos negativos diminuindo o rendimento e a qualidade dos frutos (TRINGOVSKA et al., 2015). Portanto, é necessário estudar o efeito das plantas medicinais não apenas no manejo do fitonematoide, mas também na produção da cultura principal, permitindo escolher espécies que sejam eficientes nas duas coisas. E além destes dois importantes fatores, através do cultivo de plantas medicinais os agricultores também podem obter uma segunda fonte de

renda, sendo possível obter renda maior cultivando plantas medicinais do que cultivando outras culturas comerciais e tradicionais (SINGH et al., 2019).

2.5.1 Alho (*Allium sativum*)

O alho (*Allium sativum* L.) é uma planta medicinal milenar utilizada no combate a infecções, na prevenção ao câncer, apresenta capacidade de baixar a pressão arterial e o colesterol, além de ser empregada como antibiótica, antifúngica, antiviral, anti-helmíntica, antidiabética, antioxidante, hepatoprotetora, anti-inflamatória e na cicatrização de feridas (BORLINGHAUS et al., 2014; CORZO-MARTÍNEZ; CORZO; VILLAMIEL, 2007; LONDHE et al., 2011; YEH; LIU, 2001). Suas propriedades medicinais, assim como o seu uso para fins alimentícios e agrícolas no controle de pragas e doenças estão relacionados à sua composição fitoquímica, destacando-se os compostos organosulfurados como a alicina e os tiosulfinatos (ALRUMAIHI, 2020; BORLINGHAUS et al., 2014).

O alho vem sendo utilizado no controle de nematoide-das-galhas devido à presença de substâncias químicas com propriedades nematicidas em seus bulbilhos (EDER et al., 2021; JARDIM et al., 2020; KOUAMÉ, 2021). No Brasil é encontrado o registro de um nematicida a base de extrato vegetal de *Allium sativum* recomendado para o controle de nematoide-das-galhas (*Meloidogyne incognita*) e nematoide-das-lesões (*Pratylenchus brachyurus* e *P. zeae*), sendo o produto conhecido como Vigga® (AGROFIT, 2022). Seman, Awol e Mashilla (2020) avaliaram o consórcio de tomate com alho e observaram redução na intensidade de galha e na população de nematoide-das-galhas em relação ao monocultivo de tomateiro.

O plantio de alho apresenta potencial não apenas para o manejo de nematoide-das-galhas, mas também pode proporcionar uma segunda fonte de renda para o produtor, visto que o mercado brasileiro de alho atingiu cerca de 1,6 bilhões de reais em 2020, com produção de 155.700 toneladas em área colhida de 12.223 hectares (IBGE, 2022c).

2.5.2 Vinca (*Catharanthus roseus*)

Catharanthus roseus (L.) G. Don., conhecida popularmente como vinca ou vinca-de-madagascar, é uma planta de elevado interesse farmacêutico por possuir alcaloides com atividade anticâncer, destacando-se entre eles a vimblastina e a vincristina (PHAM et al., 2020). A presença de alcaloides nesta planta pode estar relacionada ao efeito do pó das folhas secas de vinca na supressão da população de *Meloidogyne incognita* em raízes de pepino

(MOSTAFA et al., 2016). São encontrados relatos de alto índice de galhas no sistema radicular da vinca apesar de ser caracterizada como uma planta má-hospedeira (DE MENDONÇA; DE ABREU MATTOS; CARNEIRO, 2017; MCSORLEY; MCGOVERN, 2000).

2.5.3 Cravo-de-defunto (*Tagetes* spp.)

O cravo-de-defunto (*Tagetes* spp.) é uma planta medicinal que apresenta atividade antioxidante, anti-inflamatória, anti-hipertensiva, analgésica, antimicrobiana (RIAZ et al., 2020). Pode ser utilizada na agricultura auxiliando no tratamento de doenças de plantas por apresentar ação fungicida, bactericida, inseticida e nematicida (RIAZ et al., 2020; SALEHI et al., 2018).

Espécies do gênero *Tagetes* são utilizadas no manejo de nematoide-das-galhas devido à presença de substâncias nematicidas em seus exsudatos naturais, destacando-se entre estas substâncias o α-tertienil (SALEM; OSMAN, 1988; SILVA; MEDEIROS; CAMPOS, 2018; WANG; MASLER; ROGERS, 2018). O cravo-de-defunto pode ser utilizado por meio do seu plantio em consórcio com a cultura principal (VERMA, 2006; XIE et al., 2016), assim como através da rotação de culturas (XIE et al., 2016), sendo sempre interessante incorporar o material vegetal no solo para favorecer o efeito do α-tertienil (SILVA; MEDEIROS; CAMPOS, 2018).

2.5.4 Mil-folhas (*Achillea millefolium*)

A mil-folhas (*Achillea millefolium* L.) é espécie medicinal que apresenta atividade anti-inflamatória, colerética (estimula a produção de bile), antimalárica, entre outras (AKRAM, 2013). O óleo essencial obtido de suas partes aéreas apresenta atividade nematicida contra *Meloidogyne incognita*, podendo ser utilizado no controle deste nematoide (NTALLI et al., 2011). Na literatura são encontrados trabalhos destacando a mil-folhas como espécie má-hospedeira ou resistente à *Meloidogyne incognita* (EHWAETI et al., 1999) e *M. javanica* (MACIEL; FERRAZ, 1996).

2.6 Produtos biológicos no manejo de *Meloidogyne* spp.

Os bionematicidas possuem mercado emergente em todo o mundo, atingindo em 2019/2020 cerca de 82% do mercado total de nematicidas no Brasil (MACHADO, 2022), atendendo o nicho de mercado que busca estratégias de manejo com menor impacto ao ambiente e, consequentemente, mais sustentáveis. Apesar disso, ainda são enfrentados desafios em relação à confiança dos agricultores no uso de nematicidas biológicos, sendo imprescindível o seu uso respeitando as recomendações do fabricante quanto a dose, forma e período de aplicação para que o resultado seja alcançado (ABD-ELGAWAD; ASKARY, 2020; MACHADO, 2022; WILSON; JACKSON, 2013). Ademais, outro desafio enfrentado no campo é a compreensão das interações dos agentes de controle biológico com o microbioma do solo, podendo afetar espécies benéficas naquele ecossistema, ou ser afetado por outras espécies (predadores ou competidores) reduzindo sua eficácia (ABD-ELGAWAD; ASKARY, 2020). Portanto, estudos sobre os fatores que podem afetar a eficácia dos nematicidas biológicos são importantes para aumentar a confiança no seu uso.

No Brasil existem atualmente diversos produtos biológicos disponíveis no mercado para o controle de fitonematoides, sendo observados 47 nematicidas microbiológicos disponíveis no AGROFIT em Janeiro de 2022. Foram observadas 11 diferentes espécies de microrganismos nestas formulações, sendo compostas em sua maioria por uma única espécie de bactéria ou de fungo, mas também foram encontradas formulações a base de misturas com mais de uma espécie de bactérias e fungos. Filtrando as buscas no AGROFIT pela doença, para "*Meloidogyne incognita*" estão disponíveis 32 nematicidas microbiológicos e para "*Meloidogyne javanica*" estão disponíveis 30 nematicidas microbiológicos, encontrando principalmente produtos a base de bactérias do gênero *Bacillus* (AGROFIT, 2022).

2.6.1 *Bacillus subtilis*

Bactérias do gênero *Bacillus* são capazes de promover crescimento em plantas e apresentam diversos mecanismos de antagonismo a fitopatógenos, entre eles os nematoides parasitas de plantas, despertando interesse na agricultura (ENGELBRECHT et al., 2018; LANNA FILHO; FERRO; PINHO, 2010). Ao colonizar as raízes, essas bactérias podem formar biofilmes nos pontos de exsudação e atuar como agentes rizosféricos degradando substratos de origem orgânica, promovendo fixação de nitrogênio e solubilização de fosfatos (CHEN et al., 2007; CHOUDHARY; JOHRI, 2009; CORRALES RAMÍREZ et al., 2017;

KUMAR; DUBEY; MAHESHWARI, 2012). Podem atuar competindo com os fitopatógenos por recursos, além de serem capazes de produzir substâncias antagonistas a fitopatógenos e induzir mecanismos de resistência na planta, entre outras formas de controle biológico (CHAURASIA et al., 2005; CHEN et al., 2007; CHOUDHARY; JOHRI, 2009; ENGELBRECHT et al., 2018; GOND et al., 2015; HUANG et al., 2012; KUMAR; DUBEY; MAHESHWARI, 2012). O gênero *Bacillus* apresenta a vantagem de formar endósporos, possibilitando a sua manutenção e sobrevivência em habitats específicos, permitindo sua distribuição por locais com condições ambientais variáveis e favorecendo a sua aplicação na agricultura como agente de controle biológico (CORRALES RAMÍREZ et al., 2017; ENGELBRECHT et al., 2018; LANNA FILHO; FERRO; PINHO, 2010).

Diversos estudos vêm confirmando a eficácia de isolados de *Bacillus subtilis* no controle de *Meloidogyne incognita* (ADAM; HEUER; HALLMANN, 2014; BASYONY; ABO-ZAID, 2018; CAO et al., 2019; HUSSAIN et al., 2020) e *Meloidogyne javanica* (CHINHEYA; YOBO; LAING, 2017; DAWAR; TARIQ; ZAKI, 2008; SIDDIQUI, 2002). Os isolados dessa bactéria podem atuar como bionematicidas (HUSSAIN et al., 2020), matando os juvenis de segundo estádio (J2) e inibindo a eclosão de J2s (CAO et al., 2019; DAWAR; TARIQ; ZAKI, 2008), reduzindo a população deste nematoide no solo. Também podem auxiliar no controle do fitonematoide de forma indireta, promovendo indução de resistência nas plantas (ADAM; HEUER; HALLMANN, 2014).

2.7 Agricultura de precisão na detecção e monitoramento de *Meloidogyne* spp.

De acordo com a Sociedade Internacional de Agricultura de Precisão (ISPA), agricultura de precisão é uma estratégia de gestão de áreas produtivas que considera a variabilidade temporal e espacial destas áreas para melhorar a sustentabilidade da produção agrícola. Para isso, dados temporais, individuais e espaciais das áreas produtivas são coletados, reunidos, processados e analisados para extrair informações, as quais são combinadas com outras informações para apoiar as decisões de gestão da lavoura de acordo com a variabilidade estimada, aumentando a eficiência no uso de insumos e recursos, proporcionando aumento na produtividade, rentabilidade e sustentabilidade da produção agropecuária, produzindo produtos de alta qualidade e satisfazendo a demanda crescente por alimentos (ISPA, 2022; ONYANGO et al., 2021).

O desenvolvimento de ferramentas para reduzir o trabalho e o tempo gasto no monitoramento das lavouras no campo sempre despertou o interesse dos agricultores. A

utilização de imagens aéreas no manejo de campos agrícolas vem ganhando cada vez mais espaço na rotina do campo. Essas imagens fornecem dados sobre o campo de cultivo. Analisando esses dados e extraindo as informações de interesse é possível delinear zonas de manejo e construir diretrizes de cultivo de acordo com as necessidades das plantas em cada zona. Dessa forma, os agricultores podem verificar o crescimento e o estado nutricional das plantas, identificar a melhor época de colheita, detectar algumas pragas e doenças, estando preparados para realizar a agricultura de precisão (BARBEDO, 2019; RAPARELLI; BAJOCCO, 2019).

As aplicações do sensoriamento remoto usando veículos aéreos não tripulados (VANTs) vão depender do tipo de sensor que eles carregam, podendo ser câmera RGB, multispectral, hiperspectral ou termal. As câmeras RGB apresentam alta resolução espacial e baixa resolução espectral (cobrindo apenas a região do espectro visível, com as bandas vermelho, verde e azul), enquanto as câmeras multispectrais, além de alta resolução espacial, apresentam alta resolução espectral (constituídas por um conjunto de sensores, cada um sensível a determinada região espectral, geralmente cobrindo a região do espectro visível e infravermelho próximo) (MAES; STEPPE, 2019; TSOUROS et al., 2019). As câmeras hiperspectrais também apresentam alta resolução espacial e são capazes de cobrir todo o espectro eletromagnético (mais comumente na região espectral de 400 a 1000 nm) (MAES; STEPPE, 2019; TSOUROS et al., 2019). Por fim, as câmeras termais apresentam baixa resolução espacial e espectral, cobrindo apenas a região do infravermelho de ondas longas, detectando a temperatura do dossel (MAES; STEPPE, 2019; TSOUROS et al., 2019). Nesse contexto, as câmeras RGB são mais indicadas para a avaliação do vigor de crescimento das plantas, enquanto as câmeras multispectrais são indicadas para previsão de rendimento da cultura e as câmeras multi- e hiperspectrais são indicadas para avaliar o estado nutricional das plantas (MAES; STEPPE, 2019).

As câmeras RGB apresentam a desvantagem de não apresentar ferramentas para padronizar a luminosidade no momento da captura das imagens no campo, não permitindo a comparação de imagens obtidas em diferentes vôos com VANT. Alguns dos pesquisadores que usaram imagens obtidas de câmera RGB para determinar o teor de nitrogênio e a concentração de clorofila em cultivos de tomateiro (MERCADO-LUNA et al., 2010) e alface (ODABAS et al., 2017) em casa de vegetação usaram a câmera RGB acoplada a um suporte com fonte de luz padronizada para capturar as imagens. Porém, no campo não é possível realizar a padronização de luminosidade desta forma para capturar as imagens usando câmeras RGB, não permitindo a comparação de imagens RGB obtidas em diferentes vôos.

Assim, para poder comparar imagens aéreas obtidas em condições meteorológicas diferentes, a captura destas imagens em campo requer a calibração da luminosidade através do uso de painel e câmera multiespectral com sensor de incidência de luz (HUNT; DAUGHTRY, 2018). Câmeras multiespectrais têm sido usadas para calcular índices de vegetação e avaliar a saúde das plantas de alface (CUCHO-PADIN et al., 2020; REN; TRIPATHI; LI, 2017).

Na detecção de patógenos, as câmeras RGB, multi- e hiperspectrais podem ser utilizadas na identificação da severidade da infecção, enquanto as câmeras hiperspectrais e termais são capazes de detectar a doença em estágios iniciais (MAES; STEPPE, 2019). Portanto, imagens aéreas podem ser usadas para investigar e monitorar áreas infestadas por nematoides, como relatado para as culturas de café (De Abreu Júnior et al., 2020; Oliveira et al., 2019a, 2019b) e soja (Arantes et al., 2021). Buscando simplificar o processo de monitoramento e detecção da ocorrência de nematoides-das-galhas na cultura da alface e reduzir as perdas, imagens obtidas de VANTs podem ser utilizadas para avaliar as plantas em campos infestados com *M. incognita* fornecendo informações valiosas para a tomada de decisões pelos agricultores nos tratos culturais e no manejo do patógeno.

3 CONSIDERAÇÕES GERAIS

Com base nos resultados obtidos, fazer o cultivo de alface ou tomateiro em consórcio com plantas medicinais (vinca, alho, mil-folhas e cravo-de-defunto) foi eficiente em reduzir a população total de *Meloidogyne javanica* quando realizado em vaso. As plantas medicinais apresentam diferentes estratégias de resistência ao nematoide-das-galhas. Os exudatos radiculares de alho e mil-folhas não foram atrativos para os juvenis de segundo estádio (J2) de *M. javanica*, enquanto as raízes e exudatos radiculares de vinca e cravo-de-defunto apresentaram atratividade para os J2 de *M. javanica*, porém, apesar de atrativas, estas plantas foram capazes de afetar a penetração e o desenvolvimento dos J2 em suas raízes.

Realizar o cultivo de alho em consórcio com alface, aplicar *B. subtilis* BV09, fazer o pousio e eliminar as raízes infestadas foram estratégias eficientes na redução da população de *M. incognita* no campo. A produção por planta de alho foi maior ao realizar o consórcio com alface e aplicação de *B. subtilis* BV09, enquanto a produção por planta de alface foi afetada pelas plantas de alho em seu terceiro ciclo de cultivo, quando as plantas de alho estavam maiores e sombrearam a alface. A competição entre alface e alho ao final do consórcio afetou o teor de alguns nutrientes foliares nestas culturas. O consórcio possibilitou a obtenção de alta renda total das culturas de alface e alho, assim como a renda total obtida apenas com o

monocultivo de alface. O ataque de *M. incognita* ativou a resposta bioquímica de defesa das plantas, enquanto a aplicação de *B. subtilis* BV09 e o sistema de consórcio auxiliaram na proteção das plantas de alface e alho contra o estresse causado pela infecção por *M. incognita*.

A aplicação de *B. subtilis* BV09 provocou alterações na composição química dos exsudatos radiculares de alface e alho e afetou a resposta quimiotatica de *M. incognita* a eles. Na presença de *B. subtilis* BV09, os exsudatos radiculares de alface deixaram de ser atrativos e passaram a ser repelentes para *M. incognita*. Enquanto os exsudatos radiculares de alho deixaram de ser repelentes e passaram a ser atrativos para *M. incognita* na presença de *B. subtilis* BV09.

As imagens aéreas obtidas com veículo aéreo não tripulado (VANT) permitiram a avaliação do crescimento das plantas de alface ao longo do tempo, apresentando potencial para uso na detecção de áreas afetadas por nematoide-das-galhas quando as plantas apresentam seu crescimento reduzido.

REFERÊNCIAS

ABD-ELGAWAD, M. M. M. Optimizing Safe Approaches to Manage Plant-Parasitic Nematodes. **Plants**, v. 10, n. 9, p. 1911, 14 set. 2021.

ABD-ELGAWAD, M. M. M.; ASKARY, T. H. Factors affecting success of biological agents used in controlling the plant-parasitic nematodes. **Egyptian Journal of Biological Pest Control**, v. 30, n. 1, p. 17, 24 dez. 2020.

ADAM, M.; HEUER, H.; HALLMANN, J. Bacterial Antagonists of Fungal Pathogens Also Control Root-Knot Nematodes by Induced Systemic Resistance of Tomato Plants. **PLoS ONE**, v. 9, n. 2, p. e90402, 28 fev. 2014.

AGROFIT. **Sistema de Agrotóxicos Fitossanitários**. Disponível em: <https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons>. Acesso em: 20 jan. 2022.

AKRAM, M. Minireview on Achillea millefolium Linn. **The Journal of Membrane Biology**, v. 246, n. 9, p. 661–663, 20 set. 2013.

ALAMGIR, A. N. M. Cultivation of Herbal Drugs, Biotechnology, and In Vitro Production of Secondary Metabolites, High-Value Medicinal Plants, Herbal Wealth, and Herbal Trade. In: **Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1**. [s.l.] Springer International Publishing, 2017. p. 379–452.

ALI, J. G.; ALBORN, H. T.; STELINSKI, L. L. Constitutive and induced subterranean plant volatiles attract both entomopathogenic and plant parasitic nematodes. **Journal of Ecology**, v. 99, n. 1, p. 26–35, 2011.

ALRUMAIHI, F. Garlic and its Active Compounds : A Novel Strategy to Fight Diseases

Through Modulating Biological Activities. **Pharmacognosy Journal**, v. 12, n. 6, p. 1463–1474, 2020.

BARBEDO, J. A Review on the Use of Unmanned Aerial Vehicles and Imaging Sensors for Monitoring and Assessing Plant Stresses. **Drones**, v. 3, n. 2, p. 40, 20 abr. 2019.

BARRIÈRE, V. et al. Lettuce cropping with less pesticides. A review. **Agronomy for Sustainable Development**, v. 34, n. 1, p. 175–198, 2014.

BASYONY, A. G.; ABO-ZAID, G. A. Biocontrol of the root-knot nematode, Meloidogyne incognita, using an eco-friendly formulation from Bacillus subtilis, lab. and greenhouse studies. **Egyptian Journal of Biological Pest Control**, v. 28, n. 1, p. 87, 20 dez. 2018.

BORLINGHAUS, J. et al. Allicin: Chemistry and Biological Properties. p. 12591–12618, 2014.

CAMPOS, H. D.; CAMPOS, V. P.; POZZA, E. A. Efeito da temperatura na multiplicação celular, no desenvolvimento embrionário e na eclosão de juvenis do segundo estádio de Meloidogyne javanica. **Summa Phytopathologica**, v. 34, n. 1, p. 29–33, 2008.

CAO, H. et al. Analysis of the activity and biological control efficacy of the Bacillus subtilis strain Bs-1 against Meloidogyne incognita. **Crop Protection**, v. 122, p. 125–135, ago. 2019.

CARVALHO, A. C. B. et al. The Brazilian market of herbal medicinal products and the impacts of the new legislation on traditional medicines. **Journal of Ethnopharmacology**, v. 212, p. 29–35, fev. 2018.

CHARCHAR, J. M.; MOITA, A. W. **Metodologia para seleção de hortaliças com resistência a nematóides: Alface/Meloidogyne spp.** Brasília: Embrapa, 2005.

CHAURASIA, B. et al. Diffusible and volatile compounds produced by an antagonistic *Bacillus subtilis* strain cause structural deformations in pathogenic fungi in vitro. **Microbiological Research**, v. 160, n. 1, p. 75–81, fev. 2005.

CHEN, X. H. et al. Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. **Nature Biotechnology**, v. 25, n. 9, p. 1007–1014, 2007.

CHINHEYA, C. C.; YOBO, K. S.; LAING, M. D. Biological control of the rootknot nematode, Meloidogyne javanica (Chitwood) using Bacillus isolates, on soybean. **Biological Control**, v. 109, p. 37–41, jun. 2017.

CHITWOOD, D. J. PHYTOCHEMICAL BASED STRATEGIES FOR NEMATODE CONTROL. **Annual Review of Phytopathology**, v. 40, n. 1, p. 221–249, set. 2002.

CHOUDHARY, D. K.; JOHRI, B. N. Interactions of *Bacillus* spp. and plants – With special reference to induced systemic resistance (ISR). **Microbiological Research**, v. 164, n. 5, p. 493–513, set. 2009.

CHOUDHARY, V.; PRASAD, L. Morpho-pathological , Genetic Variations and Population Structure of *Sclerotinia sclerotiorum*. **International journal of plant research**, v. 25, n. 1, p. 178–183, 2012.

COOK, S. M.; KHAN, Z. R.; PICKETT, J. A. The Use of Push-Pull Strategies in Integrated Pest Management. **Annual Review of Entomology**, v. 52, n. 1, p. 375–400, 2007.

CORRALES RAMÍREZ, L. C. et al. *Bacillus* spp: una alternativa para la promoción vegetal por dos caminos enzimáticos. **Nova**, v. 15, n. 27, p. 45–65, 2017.

CORZO-MARTÍNEZ, M.; CORZO, N.; VILLAMIEL, M. Biological properties of onions and garlic. **Trends in Food Science and Technology**, v. 18, n. 12, p. 609–625, 2007.

CUCHO-PADIN, G. et al. Development of low-cost remote sensing tools and methods for supporting smallholder agriculture. **Applied Geomatics**, v. 12, n. 3, p. 247–263, 26 set. 2020.

CURTIS, R. H. C. Plant-nematode interactions: Environmental signals detected by the nematode's chemosensory organs control changes in the surface cuticle and behaviour. **Parasite**, v. 15, n. 3, p. 310–316, 2008.

D'ADDABBO, T. et al. Biocide plants as a sustainable tool for the control of pests and pathogens in vegetable cropping systems. **Italian Journal of Agronomy**, v. 9, n. 4, p. 137–145, 2014.

D'ADDABBO, T. et al. Artemisia annua compounds have potential to manage root-knot and potato cyst nematodes. **Industrial Crops and Products**, v. 108, n. March, p. 195–200, 2017.

DAWAR, S.; TARIQ, M.; ZAKI, M. J. Application of Bacillus species in control of Meloidogyne javanica (treub) chitwood on cowpea and mash bean. **Pakistan Journal of Botany**, v. 40, n. 1, p. 439–444, 2008.

DE MENDONÇA, I. C.; DE ABREU MATTOS, J. K.; CARNEIRO, R. M. D. G. Hospedabilidade de plantas medicinais a Meloidogyne paranaensis. **Nematropica**, v. 47, p. 49–54, 2017.

DELORY, B. M. et al. Root-emitted volatile organic compounds: can they mediate belowground plant-plant interactions? **Plant and Soil**, v. 402, n. 1–2, p. 1–26, maio 2016.

DONG, L. et al. Reduced Meloidogyne incognita infection of tomato in the presence of castor and the involvement of fatty acids. **Scientia Horticulturae**, v. 237, p. 169–175, 2018.

DUSENBERY, D. B. A simple animal can use a complex stimulus pattern to find a location: Nematode thermotaxis in soil. **Biological Cybernetics**, v. 60, n. 6, p. 431–437, 1989.

DUTRA, R. C. et al. Medicinal plants in Brazil: Pharmacological studies, drug discovery, challenges and perspectives. **Pharmacological Research**, v. 112, p. 4–29, 2016.

EDER, R. et al. Polysulfides Applied as Formulated Garlic Extract to Protect Tomato Plants against the Root-Knot Nematode Meloidogyne incognita. **Plants**, v. 10, n. 2, p. 394, 18 fev. 2021.

EHWAETI, M. E. et al. Host status differences and their relevance to damage by Meloidogyne incognita. **Nematology**, v. 1, n. 4, p. 421–432, 1999.

ELLING, A. A. Major emerging problems with minor Meloidogyne species. **Phytopathology**, v. 103, n. 11, p. 1092–1102, 2013.

EMBRAPA. **A cultura do tomateiro (para mesa)**. 5. ed. Brasília: EMBRAPA-SPI, 1993.

ENGELBRECHT, G. et al. Bacillus -based bionematicides: development, modes of action and commercialisation. **Biocontrol Science and Technology**, v. 28, n. 7, p. 629–653, 3 jul. 2018.

FILGUEIRAS, C. C. et al. Eliciting maize defense pathways aboveground attracts belowground biocontrol agents. **Scientific Reports**, v. 6, n. June, p. 1–9, 2016.

GOND, S. K. et al. Endophytic *Bacillus* spp. produce antifungal lipopeptides and induce host defence gene expression in maize. **Microbiological Research**, v. 172, p. 79–87, mar. 2015.

HAICHAR, F. EL Z. et al. Root exudates mediated interactions belowground. **Soil Biology and Biochemistry**, v. 77, p. 69–80, out. 2014.

HIDA, H. et al. Chemotaxis assay of plant-parasitic nematodes on a gel-filled microchannel device. **Sensors and Actuators, B: Chemical**, v. 221, p. 1483–1491, 2015.

HUANG, X. et al. Biocontrol of *Rhizoctonia solani* damping-off disease in cucumber with *Bacillus pumilus* SQR-N43. **Microbiological Research**, v. 167, n. 3, p. 135–143, mar. 2012.

HUNT, E. R.; DAUGHTRY, C. S. T. What good are unmanned aircraft systems for agricultural remote sensing and precision agriculture? **International Journal of Remote Sensing**, v. 39, n. 15–16, p. 5345–5376, 18 ago. 2018.

HUSSAIN, T. et al. Bio-nematicidal activities by culture filtrate of Bacillus subtilis HussainT-AMU: new promising biosurfactant bioagent for the management of Root Galling caused by Meloidogyne incognita. **Vegetos**, v. 33, n. 2, p. 229–238, 27 jun. 2020.

IBGE. **Produção de Alface**. Disponível em: <https://www.ibge.gov.br/explica/producao-agropecuaria/alface/br>. Acesso em: 14 jan. 2022a.

IBGE. **Produção de Tomate**. Disponível em: <https://www.ibge.gov.br/explica/producao-agropecuaria/tomate/br>. Acesso em: 14 jan. 2022b.

IBGE. **Produção de Alho**. Disponível em: <https://www.ibge.gov.br/explica/producao-agropecuaria/alho/br>. Acesso em: 14 jan. 2022c.

ISMAIL, A. E. Feasibility of growing Moringa oleifera as a mix-crop along with tomato for control of Meloidogyne incognita and Rotylenchulus reniformis in Egypt. **Archives of Phytopathology and Plant Protection**, v. 46, n. 12, p. 1403–1407, 2013.

ISPA. **Precision Ag Definition**. Disponível em: <https://www.ispag.org/about/definition>. Acesso em: 14 maio. 2022.

JAMSHIDI-KIA, F.; LORIGOOINI, Z.; AMINI-KHOEI, H. Medicinal plants: Past history and future perspective. **Journal of Herbmed Pharmacology**, v. 7, n. 1, p. 1–7, 2018.

JARDIM, I. N. et al. Garlic essential oil reduces the population of Meloidogyne incognita in tomato plants. **European Journal of Plant Pathology**, v. 157, n. 1, p. 197–209, 6 maio 2020.

KANKAM, F.; ADOMAKO, J. Influence of Inoculum Levels of Root Knot Nematodes

(Meloidogyne spp.) on Tomato (Solanum lycopersicum L.). **Asian Journal of Agriculture and Food Science**, v. 02, n. 02, p. 2321–1571, 2014.

KEBEDE, Y. et al. Unpacking the push-pull system: Assessing the contribution of companion crops along a gradient of landscape complexity. **Agriculture, Ecosystems and Environment**, v. 268, n. July, p. 115–123, 2018.

KLEIN, T. et al. Fitoterápicos: Um mercado promissor. **Revista de Ciencias Farmaceuticas Basica e Aplicada**, v. 30, n. 3, p. 241–248, 2009.

KOUAMÉ, A. P. Effectiveness of Garlic and Onion Aqueous Extracts on Tomato Root-Knot Nematodes (Meloidogyne sp.) in the Autonomous District of Yamoussoukro in Central Côte d'Ivoire. **Indian Journal of Pure & Applied Biosciences**, v. 9, n. 1, p. 24–35, 28 fev. 2021.

KRATOCHVIL, R. J. et al. Evaluation of Crop Rotation and Other Cultural Practices for Management of Root-Knot and Lesion Nematodes. **Agronomy Journal**, v. 96, n. 5, p. 1419–1428, set. 2004.

KUMAR, P.; DUBEY, R. C.; MAHESHWARI, D. K. *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. **Microbiological Research**, v. 167, n. 8, p. 493–499, 2012.

LANNA FILHO, R.; FERRO, H. M.; PINHO, R. S. C. DE. Controle biológico mediado por *Bacillus subtilis*. **Revista Trópica - Ciências Agrárias e Biológicas**, v. 4, p. 12–20, 2010.

LONDHE, V. P. et al. Review Role of Garlic (*Allium Sativum*) in Various Diseases : an Overview. **Journal of Pharmaceutical Research and Opinion**, v. 4, p. 129–134, 2011.

LOURENÇO JR., V.; LOPES, C. A.; REIS, A. **Rotação e Sucessão de Culturas em Hortaliças Cultivadas em Pequenas Áreas no Manejo de Doenças**. 1. ed. Brasília: Embrapa Hortaliças, 2016.

LUPATINI, M. et al. Long-term farming systems modulate multi-trophic responses. **Science of the Total Environment**, v. 646, p. 480–490, 2019.

MACHADO, A. C. Z. Bionematicides in Brazil: an emerging and challenging market. **Revisão Anual de Patologia de Plantas**, p. 35–49, 10 jan. 2022.

MACIEL, S. L.; FERRAZ, L. C. C. B. REPRODUÇÃO DE Meloidogyne incognita RAÇA 2 E DE Meloidogyne javanica EM OITO ESPÉCIES DE PLANTAS MEDICINAIS. **Scientia Agricola**, v. 53, n. 2–3, p. 232–236, maio 1996.

MAES, W. H.; STEPPE, K. Perspectives for Remote Sensing with Unmanned Aerial Vehicles in Precision Agriculture. **Trends in Plant Science**, v. 24, n. 2, p. 152–164, fev. 2019.

MALDONADE, I. R.; MATTOS, L. M.; MORETTI, C. L. **Manual de boas práticas agrícolas na produção de alface**. Brasília: Embrapa Hortaliças, 2014.

MASHELA, P. W. et al. Interactions of Cucumis myriocarpus, Lippia javanica and Ricinus communis organic amendments on suppression of Meloidogyne incognita. **Journal of Phytopathology**, v. 155, n. 11–12, p. 690–693, 2007.

MATEUS, M. A. F. et al. Extratos aquosos de plantas medicinais no controle de Meloidogyne incognita (Kofoid e White, 1919) Chitwood, 1949. **Bioscience Journal**, v. 30, n. 3, p. 730–736, 2014.

MCSORLEY, R.; MCGOVERN, R. J. Effects of Solarization and Ammonium Amendments on Plant-Parasitic Nematodes. **Journal of Nematology**, v. 32, n. 4S, p. 537–541, 2000.

MERCADO-LUNA, A. et al. Nitrogen determination on tomato (Lycopersicon esculentum Mill.) seedlings by colour image analysis (RGB). **African Journal of Biotechnology**, v. 9, n. 33, p. 5326–5332, 2010.

MINISTÉRIO DA SAÚDE. **Uso de plantas medicinais e fitoterápicos sobe 161%**.

MOSTAFA, F. et al. Potential Use of Botanicals Rich in Alkaloids for Controlling Meloidogyne incognita and Rotylenchulus reniformis Infecting Cucurbits. **Egyptian Journal of Agronematology**, v. 15, n. 1, p. 29–43, 2016.

NAIKA, S. et al. **A cultura do tomate**. Wageningen, Países Baixos: Digigrafi, 2006.

NTALLI, N. G. et al. Synergistic and antagonistic interactions of terpenes against Meloidogyne incognita and the nematicidal activity of essential oils from seven plants indigenous to Greece. **Pest Management Science**, v. 67, n. 3, p. 341–351, mar. 2011.

ODABAS, M. S. et al. Multilayer Perceptron Neural Network Approach to Estimate Chlorophyll Concentration Index of Lettuce (Lactuca sativa L.). **Communications in Soil Science and Plant Analysis**, v. 48, n. 2, p. 162–169, 19 jan. 2017.

OLIVEIRA, C. M. G. DE; ROSA, J. M. O. **Nematoides Parasitos da Alface**. São Paulo: Secretaria de Agricultura e Abastecimento do Estado de São Paulo, 2014.

ONYANGO, C. M. et al. Precision Agriculture for Resource Use Efficiency in Smallholder Farming Systems in Sub-Saharan Africa: A Systematic Review. **Sustainability**, v. 13, n. 3, p. 1158, 22 jan. 2021.

PERRY, R. N. CHEMORECEPTION IN PLANT PARASITIC NEMATODES. **Annual Review of Phytopathology**, v. 34, n. 1, p. 181–199, set. 1996.

PHAM, H. N. T. et al. Phytochemicals Derived from Catharanthus roseus and Their Health Benefits. **Technologies**, v. 8, n. 4, p. 80, 21 dez. 2020.

PICKETT, J. A. et al. Push–pull farming systems. **Current Opinion in Biotechnology**, v. 26, p. 125–132, abr. 2014.

PICKETT, J. A.; KHAN, Z. R. Plant volatile-mediated signalling and its application in agriculture: successes and challenges. **New Phytologist**, v. 212, n. 4, p. 856–870, 2016.

PINHEIRO, J. B.; PEREIRA, R. B.; SUINAGA, F. A. **Manejo de nematoides na cultura do tomate**. 1. ed. Brasília: Embrapa Hortaliças, 2014.

RALMI, N. H. A. A.; KHANDAKER, M. M.; MAT, N. Occurrence and control of root knot nematode in crops: A review. **Australian Journal of Crop Science**, v. 10, n. 12, p. 1649–1654, 2016.

RAPARELLI, E.; BAJOCCO, S. A bibliometric analysis on the use of unmanned aerial vehicles in agricultural and forestry studies. **International Journal of Remote Sensing**, v. 40, n. 24, p. 9070–9083, 17 dez. 2019.

RASMANN, S. et al. Ecology and Evolution of Soil Nematode Chemotaxis. **Journal of Chemical Ecology**, v. 38, n. 6, p. 615–628, 30 dez. 2012.

RATNADASS, A. et al. Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. **Agronomy for Sustainable Development**, v. 32, n. 1, p. 273–303, 30 dez. 2012.

REN, D. D. W.; TRIPATHI, S.; LI, L. K. B. Low-cost multispectral imaging for remote sensing of lettuce health. **Journal of Applied Remote Sensing**, v. 11, n. 1, p. 016006, 11 jan. 2017.

RIAZ, M. et al. Traditional uses, Phyto-chemistry and pharmacological activities of Tagetes Patula L. **Journal of Ethnopharmacology**, v. 255, p. 112718, jun. 2020.

ROCHA, F. S.; CAMPOS, V. P. Efeito de exsudatos de cultura de células de plantas em juvenis de segundo estádio de Meloidogyne incognita. **Fitopatologia Brasileira**, v. 29, n. 3, p. 294–299, jun. 2004.

ROVIRA, A. D. Plant root exudates. **The Botanical Review**, v. 35, n. 1, p. 35–57, 1969.

SALEHI, B. et al. Tagetes spp. Essential Oils and Other Extracts: Chemical Characterization and Biological Activity. **Molecules**, v. 23, n. 11, p. 2847, 1 nov. 2018.

SALEM, F. M.; OSMAN, G. Y. Effectiveness of tagetes natural exudates onMeloidogyne javanica (Chitwood) nematode. **Anzeiger für Schädlingskunde Pflanzenschutz Umweltschutz**, v. 61, n. 1, p. 17–19, 30 dez. 1988.

SANTOS, H. P. DOS et al. **Princípios básicos da consorciação de culturas**. Passo Fundo: Embrapa Trigo, 2007.

SEID, A. et al. Tomato (Solanum lycopersicum) and root-knot nematodes (Meloidogyne spp.)-a century-old battle. **Nematology**, v. 17, n. 9, p. 995–1009, 2015.

SEMAN, A.; AWOL, S.; MASHILLA, D. Integrated management of Meloidogyne incognita in tomato (Solanum lycopersicum) through botanical and intercropping. **African Journal of Agricultural Research**, v. 15, n. 4, p. 492–501, 30 abr. 2020.

SIDDIQUI, I. A. Suppression of Meloidogyne javanica by Pseudomonas aeruginosa and Bacillus subtilis in tomato. **Nematologia Mediterranea**, v. 30, n. 2, p. 125–130, 2002.

SIKORA, R. A.; FERNANDEZ, E. Nematode parasites of vegetables. In: LUC, M.; SIKORA, R. A.; BRIDGE, J. (Eds.). . **Plant-parasitic nematodes in subtropical and tropical agriculture**. 2. ed. Wallingford, UK: CAB International, 2005. p. 319–391.

SILVA, J. C. P. DA; MEDEIROS, F. H. V. DE; CAMPOS, V. P. Building soil suppressiveness against plant-parasitic nematodes. **Biocontrol Science and Technology**, v. 28, n. 5, p. 423–445, 2018.

SILVA, R. G. DA et al. Crop succession and rotation with surface liming on nematode management and soybean yield. **Pesquisa Agropecuária Brasileira**, v. 57, 2022.

SILVA, J. DE O. **Meloidogyne incognita NA CULTURA DO TOMATE: LEVANTAMENTO E MANEJO COM PRODUTOS BIOLÓGICOS**. Goiânia: Programa de Pós-Graduação em Agronomia, 2015.

SINGH, K. K. et al. Income Generation of Farmers via Medicinal Plants Cultivation: A Review. **International Journal of Pure & Applied Bioscience**, v. 7, n. 4, p. 174–177, 5 out. 2019.

SINGH, R. Medicinal plants: A review Singh. **Journal of Plant Sciences**, v. 3, n. 1, p. 50–55, 2015.

SINGH, S.; SINGH, B.; SINGH, A. P. Nematodes: A Threat to Sustainability of Agriculture. **Procedia Environmental Sciences**, v. 29, p. 215–216, 2015.

SOM, S.; WILLETT, D. S.; ALBORN, H. T. Dynamics of belowground volatile diffusion and degradation. **Rhizosphere**, v. 4, n. July, p. 70–74, 2017.

STEVENSON, P. C.; ISMAN, M. B.; BELMAIN, S. R. Pesticidal plants in Africa: A global vision of new biological control products from local uses. **Industrial Crops and Products**, v. 110, n. August, p. 2–9, 2017.

SUBEDI, S.; THAPA, B.; SHRESTHA, J. Root-knot nematode (Meloidogyne incognita) and its management: a review. **Journal of Agriculture and Natural Resources**, v. 3, n. 2, p. 21–31, 30 out. 2020.

TAMASI, G. et al. Characterization of nutraceutical components in tomato pulp, skin and locular gel. **European Food Research and Technology**, v. 245, n. 4, p. 907–918, 17 abr. 2019.

TORTO, B. et al. Management of Cyst and Root Knot Nematodes: A Chemical Ecology Perspective. **Journal of Agricultural and Food Chemistry**, v. 66, n. 33, p. 8672–8678, 2018a.

TORTO, B. et al. Strategies for the Manipulation of Root Knot Nematode Behavior with Natural Products in Small Scale Farming Systems. **ACS Symposium Series**, v. 1294, p. 114–126, 2018b.

TRINGOVSKA, I. et al. Effect of companion plants on tomato greenhouse production. **Scientia Horticulturae**, v. 186, p. 31–37, 2015.

TSOUROS, D. C. et al. Data Acquisition and Analysis Methods in UAV- based Applications for Precision Agriculture. In: **15th International Conference on Distributed Computing in Sensor Systems (DCOSS)**. [s.l.] IEEE, 2019. p. 377–384.

VALE, F. X. R.; LOPES, C. A.; ALVARENGA, M. A. R. Manejo integrado das doenças do tomateiro: epidemiologia e controle. In: ALVARENGA, M. A. R. (Ed.). . **Tomate: produção em campo, em casa de vegetação e em hidroponia**. Lavras: Editora UFLA, 2004. p. 400.

VERMA, K. K. Management of root-knot nematode (Meloidogyne javanica) in fieldpea

(Pisum sativum) by intercropping with marigold (Tagetes erecta). **Annals of Agri Bio Research**, v. 11, n. 2, p. 121–122, 2006.

VESTERGÅRD, M. Trap crops for Meloidogyne hapla management and its integration with supplementary strategies. **Applied Soil Ecology**, v. 134, n. August 2018, p. 105–110, 2019.

WANG, C.; BRUENING, G.; WILLIAMSON, V. M. Determination of preferred pH for root-knot nematode aggregation using pluronic F-127 Gel. **Journal of Chemical Ecology**, v. 35, n. 10, p. 1242–1251, 2009.

WANG, C.; MASLER, E. P.; ROGERS, S. T. Responses of Heterodera glycines and Meloidogyne incognita Infective Juveniles to Root Tissues, Root Exudates, and Root Extracts from Three Plant Species . **Plant Disease**, v. 102, n. 9, p. 1733–1740, 2018.

WILSON, M. J.; JACKSON, T. A. Progress in the commercialisation of bionematicides. **BioControl**, v. 58, n. 6, p. 715–722, 9 dez. 2013.

XIE, G.-H. et al. Crop rotation and intercropping with marigold are effective for root-knot nematode (Meloidogyne sp.) control in angelica (Angelica sinensis) cultivation. **Canadian Journal of Plant Science**, v. 97, n. 1, p. 26–31, 2016.

YEH, Y.-Y.; LIU, L. Cholesterol-Lowering Effect of Garlic Extracts and Organosulfur Compounds: Human and Animal Studies. **The Journal of Nutrition**, v. 131, n. 3, p. 989S-993S, abr. 2001.

YOKORO, G. K.; PEREIRA, J. A. Produção e comercialização de alface: um estudo a partir da perspectiva dos produtores do município de Naviraí/MS. **Revista Agropampa**, v. 3, n. 3, p. 64–79, 2020.

ZAKER, M. Natural Plant Products as Eco-friendly Fungicides for Plant Diseases Control- A Review. **The Agriculturists**, v. 14, n. 1, p. 134–141, 2016.

SEGUNDA PARTE – ARTIGOS

ARTIGO 1

Intercropped medicinal plants reduced *Meloidogyne javanica* population by affecting its attraction to crop roots

(versão preliminar submetida ao periódico: Plant and Soil)

ARTIGO 2

Integrated root-knot nematode management on lettuce crops using cultural and biological practices: garlic cultivation and *Bacillus subtilis* BV09 application

(versão preliminar submetida ao periódico: Agricultural Systems)

ARTIGO 3

Bacillus subtilis BV09 affecting root exudates metabolomics and *Meloidogyne incognita* chemotactic response

(versão preliminar submetida ao periódico: Plant Physiology and Biochemistry)

ARTIGO 4

Use of RGB images from unmanned aerial vehicle to estimate lettuce growth in root-knot nematode infested soil

(publicado em: Smart Agricultural Technology)

CARTILHA DE ORIENTAÇÃO PARA OS PRODUTORES

Manejo integrado de nematoide-das-galhas na cultura da alface

Versão preliminar submetida ao periódico: Plant and Soil

Intercropped medicinal plants reduced *Meloidogyne javanica* population by affecting its attraction to crop roots

ABSTRACT

Background and Aims

The application of antagonistic plants by intercropping is a feasible practice to reduce root-knot nematodes (RKN). Many medicinal plants are chemically antagonistic to RKN. Here, medicinal species were tested as possible intercrops to be planted near tomato or lettuce to reduce the RKN *Meloidogyne javanica* population, and the intercrops' exudates chemotaxis to RKN was verified.

Methods

Garlic (*Allium sativum*), Madagascar periwinkle (MP) (*Catharanthus roseus*) and yarrow (*Achillea millefolium*) were used as intercrops. Marigold (*Tagetes patula*) and tomato were used as positive and negative control, respectively. The root attractiveness was evaluated by growing the seedlings on agar medium. Extracted root exudates of each plant were tested on agar medium. To investigate the effects of cultivating lettuce or tomato intercropped with medicinal plants on *M. javanica* population, two plant species were grown side by side in a pot, simulating the intercropping.

Results

Higher attractiveness was observed by tomato and marigold. Higher root penetration occurred in tomato, while MP and marigold significantly reduced the second-stage juvenile (J2) penetration. The J2 inside MP and marigold roots failed to reach the adult stage. The most attractive exudates came from tomato and marigold followed by lettuce and MP. The total number of *M. javanica* eggs was lower when tomato or lettuce were grown with medicinal plants compared to only lettuce or tomato. The shoot growth of both plants significantly improved when intercropped with MP, yarrow, or garlic.

Conclusion

Therefore, MP, yarrow and garlic were favorable intercrops able to affect *M. javanica* attraction, reduce its population and improve plant growth.

Keywords: root-knot nematode; antagonistic plants; chemotaxis; management; root exudates.

1. Introduction

Plant-parasitic nematodes are microscopic organisms that highly damage a great number of crops over the world (Singh et al. 2015; Ralmi et al. 2016). The species of the genus *Meloidogyne*, known as root-knot nematodes (RKN), have a wide range of hosts with *Meloidogyne javanica* being one of the most distributed species worldwide (Sikora and Fernandez 2005). Among the hosts are tomato (*Solanum lycopersicum* L.) and lettuce (*Lactuca sativa* L.), where 10 to 100% of the production can be lost due to *Meloidogyne* spp. attack (Seid et al. 2015; Phani et al. 2021; Mendoza-De Gives 2022).

The control of RKN is too costly, and their eradication is practically impossible, making it necessary to implement a set of successful control practices, such as the use of healthy seedlings, cropping resistant cultivars, eliminating host weeds, manipulation of fallow areas, biological control, use of non-host plants in crop rotation and intercropping with species that have an antagonistic effect on RKN (Collange et al. 2011; Sivasubramaniam et al. 2020; Mendoza-De Gives 2022).

There are different practices to manage RKN by cultivating antagonistic plants (Oka 2010; Sivasubramaniam et al. 2020). The intercropping practice is based on the replacement of a major crop, usually susceptible to RKN, by an antagonist species, by performing a polyculture system, which requires the use of antagonistic plants with a longer cycle than the major crop (Rodríguez-kábana and Canullo 1992; Torto et al. 2018b; Homulle et al. 2022). It is known that in natural ecosystems plants always look for propitious conditions to establish themselves and exclude other competitors (Wilschut et al. 2017). However, the lack of co-evolution by entering different plant species may lead to novel plant-enemy interactions and consequently affect pathogenic invasions by changing the chemistry involved in the interaction (Verhoeven et al. 2009). The intercropping process established in agricultural systems brings this aspect to a profitable approach in agricultural fields (Maitra et al. 2019).

In addition to the excluding aspect of other competing plants in the natural ecosystem, the intercropping system directly affects the growth of the major crops (Zhou et al. 2011; Tringovska et al. 2015) as well as infestation by RKN, where the different plant will provide different host suitability (Marques et al. 2012). The infective stage of RKN, the second stage juveniles (J2), present sensorial organs that fastly respond to root exudate compounds and can promptly migrate and penetrate the host roots. Therefore, root exudation is the first and the major player to mediate the host-attraction-repellence process for RKNs, which will determine the success or failure of increasing the population (Wilschut et al. 2017; Murungi et al. 2018).

Medicinal plants are profitable alternative crops used for different therapy ends and their pharmacological actions depend on their phytochemical composition (A. Hussein and A. El-Anssary 2019). The complexity of compounds from medicinal plants may affect pests and pathogens (Mateus et al. 2014; D'Addabbo et al. 2017; Silva et al. 2020) or also interfere with nematode migration (Ali et al. 2011; Filgueiras et al. 2016; Torto et al. 2018a). For example, garlic (*Allium sativum*) has nematicidal compounds effective against Meloidogyne incognita in its essential oil or extracts (Jardim et al. 2020; Eder et al. 2021; Kouamé 2021), Madagascar periwinkle (*Catharanthus roseus*) and yarrow (*Achillea millefolium*) are non-host plants to *Meloidogyne* spp. (Maciel and Ferraz 1996; McSorley and McGovern 2000; de Mendonça et al. 2017), and marigold species (*Tagetes* spp.) have nematicidal compounds and are already used in crop rotation or intercropping for the management of some RKN (Xie et al. 2016; Long et al. 2019).

In this way, root attractiveness, and the ability of J2 to penetrate and develop in the roots are relevant parameters to evaluate in an intercropping system for RKN management. Medicinal plants intercropped with other commercial and traditional crops may improve the system incomes and assists the RKN management. Thus, this study aimed to evaluate

Meloidogyne javanica attraction by different medicinal plant species and the RKN population behavior in intercropped systems of lettuce or tomato with medicinal plants by pot experiments.

2. Materials and Methods

Laboratory and greenhouse experiments were conducted to evaluate medicinal plants' resistance mechanisms to *M. javanica*, and to verify the effect of the intercropping of tomato and lettuce with the selected medicinal plants in the management of *M. javanica* (Fig. 1).

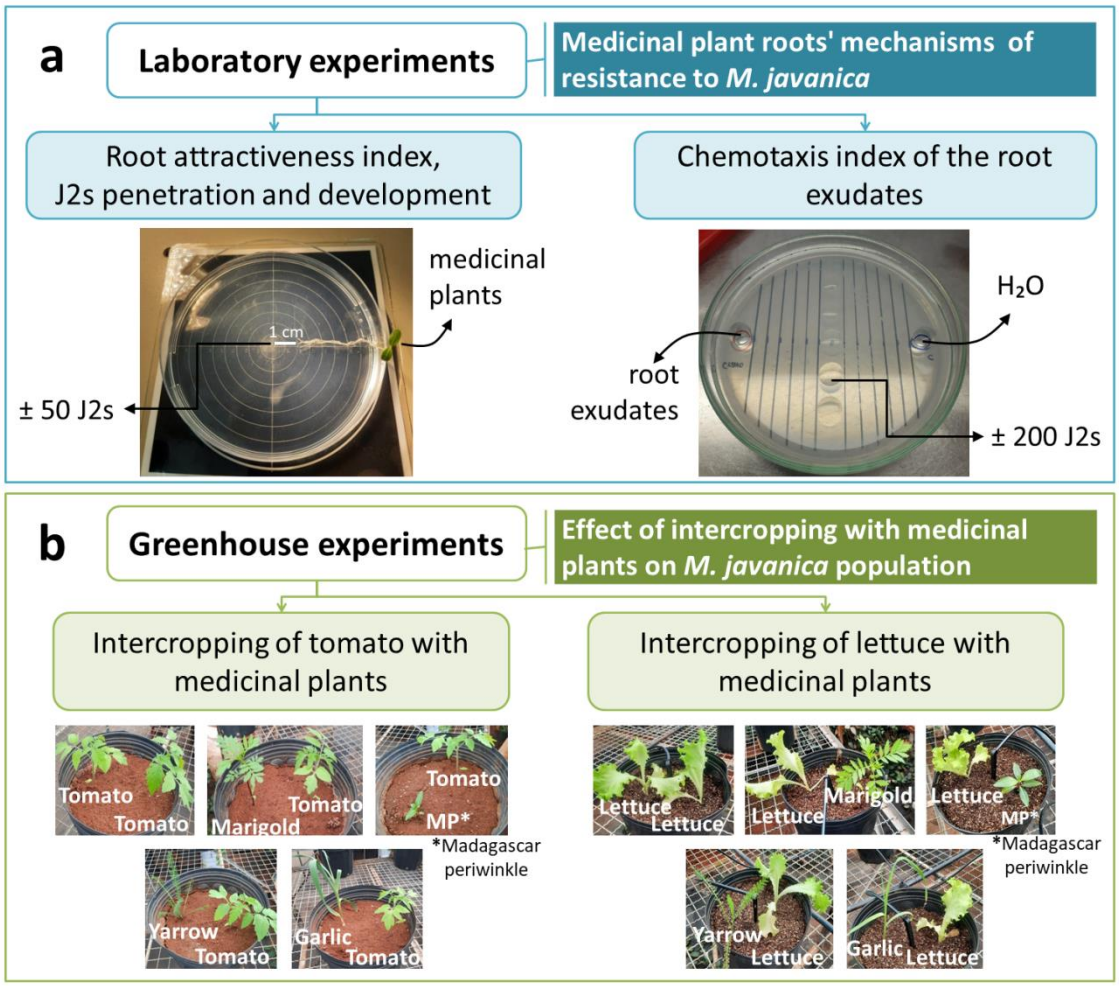

Fig. 1 Laboratory (**a**) and greenhouse (**b**) experiments to evaluate the resistance mechanisms of the selected medicinal plants and the effect of intercropping tomato and lettuce crops with these plants in the management of *Meloidogynejavanica*. *J2s: second stage juveniles. Laboratory experiments were adapted from Dong et al. (2014), Wilschut et al. (2017) and Wang et al. (2019).

99

100 2.1 Obtaining *Meloidogyne javanica* population

101 The RKN *M. javanica* (Treub) Chitwood was selected due to its large number of host
102 plants and worldwide distribution (Sikora and Fernandez 2005; Pinheiro et al. 2014). The *M.*
103 *javanica* population was previously established in tomato plants (*Solanum lycopersicum* L.
104 cultivar 'Santa Clara') kept in a greenhouse at the Federal University of Lavras (UFLA),
105 Brazil.

106 Eggs were extracted by cutting roots into small pieces and stirring in a blender with
107 0.5% NaOCl for 40 seconds. The grind root suspension was passed through a 0.075 mm (200
108 mesh) sieve coupled to a 0.025 mm opening (500 mesh), in which the eggs were collected and
109 rinsed under running water (Hussey and Barker 1973). To obtain second-stage juveniles (J2),
110 the eggs were placed in hatching chambers by Baermann funnels, and only freshly collected
111 J2 was used in the experiments.

112

113 2.2 Choice and acquisition of medicinal plants

114 The medicinal plants were selected based on studies regarding the potential species for
115 nematode control. Garlic (*Allium sativum* L.) was selected due to the presence of nematicidal
116 compounds effective against *Meloidogyne incognita* in its essential oil or extracts (Jardim et
117 al. 2020; Eder et al. 2021; Kouamé 2021). Madagascar periwinkle (*Catharanthus roseus* (L.)
118 G. Don.) was selected due to reports of this plant being a non-host to *M. incognita* and *M.*
119 *paranaensis*, despite having a high rate of galls in its root system (McSorley and McGovern
120 2000; de Mendonça et al. 2017), suggesting that it is an attractive plant to RKNs. Yarrow
121 (*Achillea millefolium* L.) was selected because it is a non-host of *M. javanica* (Maciel and
122 Ferraz 1996). Marigold (*Tagetes patula* L.) was chosen as the positive control because it is

123 widely used in the management of RKN by producing nematicidal compounds in its roots
124 exudation (Salem and Osman 1988; Verma 2006; Xie et al. 2016; Wang et al. 2018).

125 Marigold and Madagascar periwinkle were obtained from commercial seeds. Garlic
126 and yarrow were obtained through vegetative propagation. The garlic bulbs were obtained
127 from the horticulture branch of the Crop Science Department of UFLA, and the yarrow
128 seedlings were grown in the Medicinal Plants Garden of UFLA.

129

130 2.3 Laboratory experiments

131 *2.3.1 Roots attractiveness index, penetration and development of Meloidogyne javanica*

132 The assay was performed according to Dong et al. (2014) and Wilschut et al.(2017)
133 with minor adaptations. Garlic bulbs and seeds of sucetible tomato cultivar 'Santa Clara',
134 Madagascar periwinkle and marigold were disinfected for one minute in a water solution of
135 70% ethanol and 10 minutes in a water solution of 0.75% hypochlorite, followed by triple
136 washing in distilled water. Then, they were planted in autoclaved vermiculite. After 10 days
137 of growth, the roots were washed in distilled water and placed on the surface of sterile agar–
138 water medium (0.5%, 5 g/L) in Petri dishes (9 cm), leaving the shoots standing outside. The
139 dishes were kept in a growth chamber at 25°C with a 12-hour photoperiod. After five days,
140 approximately 50 J2 of *M. javanica* were applied on the surface of the medium 1 cm away
141 from the roots (Fig. 1**a**), and the dishes were kept at room temperature in dark. After 4 h, the
142 number of J2s inside the 0.5 cm area around the roots was quantified using a stereoscopic
143 microscope. The number of nematodes inside the 0.5 cm area around the roots divided by the
144 total number of J2s per dish was used as an attractiveness index; values ranged between 0 and
145 1, and the more attractive the root was, the closer the index value was to 1. Each plant species
146 corresponded to a treatment. Tomato was used as a control in this study.

Approximately 17 days after adding the J2s to the dishes, the nematodes in the roots of the plants were stained following a methodology using food dye (Rocha et al. 2005). Then, the number of J2s that penetrated the roots was quantified, and the nematode development stages inside the roots were observed. Images were captured using Motic Images Plus software version 2.0 and a optical microscope (Motic BA310 Series, Schertz, TX, United States) coupled to a Moticam 3.0 MP camera (Motic, Schertz, TX, United States).

2.3.2 Obtention of root exudates

The root exudates were obtained based on the methodology described by Čepulyteet al. (2018) and Liu et al. (2019). Tomato (*S. lycopersicum* cultivar 'Santa Clara'), lettuce (*L. sativa* cultivar 'Solaris'), marigold (*T. patula*), Madagascar periwinkle (*C. roseus*), yarrow (*A. millefolium*) and garlic (*A. sativum*) plants with approximately five weeks of growth were used. The roots were washed and placed in glass flasks dipped in 40 mL of sterile distilled water, leaving the shoots outside the flask. The roots of 10 plants (tomato, Madagascar periwinkle, marigold) or five plants (lettuce, yarrow and garlic) were placed in each flask.The number of plants was defined according to their root systems volumes, which were submerged in 40 mL of water. The plants were kept under these conditions for 24 hours at room temperature. Then, the water containing the root exudates was collected, filtered through filter paper and stored in conical tubes (15 mL). The tubes were stored in a refrigerator for approximately 24 hours until the samples were used.

2.3.3 Chemotaxis index

To observe the effect of the substances released (exudates) by the roots of the medicinal plants on the movement of *M. javanica*, a chemotaxis assay was performed based on the method described by Wang et al. (2019). The treatments corresponded to the root

exudates obtained (according to item 2.3.2) from each of the following plant species: tomato (*S. lycopersicum* cultivar 'Santa Clara'), lettuce (*L. sativa* cultivar 'Solaris'), marigold (*T. patula*), Madagascar periwinkle (*C. roseus*), yarrow (*A. millefolium*) and garlic (*A. sativum*), and a control (distilled water). Petri dishes 9 cm in diameter with 20 mL of agar–water medium (2%, 2 g/L) were used to observe the movement of *M. javanica* J2s (Fig. 1**a**). On both sides of the dish (test area and control area, Fig. S1), holes were made in the agar–water medium using straws of 1 cm diameter. The holes were made approximately 2.5 cm from the neutral area where the J2s were added. A total of 100 µL of root exudates was added to the hole in the test area (+), and 100 µL of distilled water was added to the hole in the control area (-). The negative control was performed by adding only water to both holes (test area and control area, Fig. S1). Approximately 200 J2s were placed in the center of the Petri dish (neutral area, Fig. S1). Then, the dishes were kept at room temperature in the dark. After 16 h, the number of J2s in the test and control areas was quantified. The chemotaxis index (CI) was calculated using the equation below:

$$\mathrm{CI} = \frac{(\text{number of J2s in the test area} - \text{number of J2s in the control area})}{\text{total number of J2s in the dish}}$$

According to Wang et al. (2019), CI values> 0.2 are considered highly attractive; CI> 0.1 but <0.2, slightly attractive; and CI> −0.1 but <0.1, a random response.

2.4 Greenhouse experiments

2.4.1 Intercropping of tomato with medicinal plants

Seedlings of tomato (*S. lycopersicum* cultivar 'Santa Clara'), marigold (*T. patula*), Madagascar periwinkle (*C. roseus*), yarrow (*A. millefolium*) and garlic (*A. sativum*), with approximately five weeks of growth, were grown under different treatments in 11 L pots filled

196 with 10 L of soil mixed with sand, sterilized by solarization for five weeks and then sieved.
197 The treatments consisted of one medicinal species (Madagascar periwinkle, yarrow and
198 garlic) intercropped with one tomato plant, presenting two plants grown per pot (Fig. 1**b**). In
199 the negative control, two tomato plants were planted per pot. Tomato cultivar 'Santa Clara'
200 was used because of its susceptible to *M. javanica* (Seid et al. 2015). Marigold was used as a
201 positive control, being intercropped with tomato as well as the other medicinal plants. The
202 experimental design was completely randomized (DIC), consisting of five treatments.

203 Before transplanting the seedlings, soil samples were collected, and the presence of
204 RKN was not detected. The chemical characterization of the soil was determined: pH = 4.84
205 ($CaCl_2$) and pH = 5.44 (H_2O); P = 1.19 mg/dm^3; K = 94.00 mg/dm^3; S = 0.87 mg/dm^3; Na =
206 0.00 mg/dm^3; Ca = 2.60 cmolc/dm^3; Mg = 0.73 cmolc/dm^3; Al = 0.04 cmolc/dm^3; t = 3.61
207 cmolc/dm^3; T = 7.67 cmolc/dm^3; aluminum saturation = 1.1%; base saturation = 46.54%;
208 organic matter = 4.37%; Cu = 8.00 mg/dm^3; Fe = 43.40 mg/dm^3; Mn = 91.10 mg/dm^3; and Zn
209 = 3.90 mg/dm^3. Two months before the beginning of the experiment, the soil pH was
210 corrected to 6.00 with limestone. One part of this soil was mixed with 2 parts of sand to
211 reduce the clay percentage from 53.5% to 17.8% and increase the sand percentage from
212 19.9% to 73.3%, since the reduction of clay and increasing sand are more favorable to the
213 development of *M. javanica* (Rinaldi et al. 2014).

214 Approximately one week after transplanting the seedlings, approximately 5,000 eggs
215 were added to small holes made in the soil around each plant, totaling 10,000 eggs per pot.
216 The plants were kept in a greenhouse covered with transparent polyethylene film (150 µm)
217 and 70% shade cloth. Irrigation was performed by drip irrigation according to plant needs.

218 The pots were fertilized before planting with simple superphosphate following the 5th
219 approach recommendation (Ribeiro et al. 1999). During the experiment, top dressing
220 fertilizers were made in a mixture with potassium nitrate and calcium nitrate or with mono

ammonium phosphate and magnesium sulfate, applying each mixture twice a week on different days. Foliar fertilization was also sprayed with potassium nitrate and amino acids, mono ammonium phosphate and magnesium sulfate, or micronutrients (Supa trace 10 - Agrichem®, Ribeirão Preto, SP, Brazil).

Approximately 80 days after transplanting and 70 days after the eggs had been added to the soil, plant height, root fresh mass and number of eggs were evaluated. The eggs were extracted as previously described by Hussey and Barker technique. The eggs were counted under a stereoscopic microscope using a Peters camera, and three counts were performed per sample.

2.4.2 Intercropping of lettuce with medicinal plants

Lettuce (*L. sativa* cultivar 'Solaris'), marigold (*T. patula*), Madagascar periwinkle (*C. roseus*), yarrow (*A. millefolium*) and garlic (*A. sativum*) seedlings, with approximately five weeks of growth, were grown under different treatments in five-liter pots filled with a commercial substrate composed of peat, vermiculite, class A agro-industrial organic waste and limestone (Carolina Soil®, Santa Cruz do Sul, RS, Brazil). The treatments consisted of one medicinal species (Madagascar periwinkle, yarrow and garlic) intercropped with one lettuce plant, presenting two plants grown per pot (Fig. 1**b**). Two lettuce plants per pot were planted in the negative control. Lettuce cultivar 'Solaris' was used because of its susceptibility to *M. javanica* (Sgorlon et al. 2018). Marigold was used as a positive control, being intercropped with lettuce as well as the other medicinal plants. The experimental design was completely randomized (DIC), consisting of five treatments.

Approximately one week after planting, approximately 1,000 J2s were added to small holes made in the substrate around each plant, totaling 2,000 J2s per pot. The plants were kept in a greenhouse covered with transparent polyethylene film (150 microns) and 70% shade

cloth. Irrigation was performed by drip irrigation according to the plant's needs. The plants were foliar fertilized with NIPHOKAM 108 (Fênix Agro, Tietê, SP, Brazil) following the manufacturer's recommendations.

Approximately 60 days after the introduction of the J2s to the substrate, the fresh mass of the aerial parts of the plants, the fresh mass of the roots and the number of eggs were evaluated. The eggs were extracted as previously described by Hussey and Barker technique. The eggs were counted under a stereoscopic microscope using a Peters camera, and three counts per replicate of each treatment were performed.

2.5 Statistical analyses

The normality of the data and the homogeneity of the variances were evaluated by the Shapiro–Wilk test and Levene test, respectively. All the experiments had five replicates. All the laboratory experiments were done twice and the values were pulled together. Then, the data were subjected to analysis of variance (ANOVA) and the means were grouped by the Scott–Knott test or compared by the Tukey test at 5% significance. Statistical analyses were performed using R software (R Core Team 2019). Data of root fresh mass from lettuce plants did not show homogeneity and it was transformed by log(x) before analysis.

3. Results

3.1 Medicinal plant roots attractiveness and *Meloidogyne javanica* penetration in the roots

M. javanica was attracted to all the plants with different levels of attractiveness (Fig. 2). Marigold and tomato roots were similarly attractive to *M. javanica.* The attractiveness index of the Madagascar periwinkle and garlic roots differed significantly ($p < 0.001$) from the index observed for the tomato root, showing low attractiveness for *M. javanica* (Fig. 2). The *M. javanica* J2s were observed around the root system of the tested plants (Fig. S2). The J2s

penetrate the roots of tomato, Madagascar periwinkle and marigold, with significant differences ($p < 0.001$). The highest percentage of J2 penetration was observed in the tomato roots. On the other hand, the penetration of the J2s in Madagascar periwinkle roots was 30 % lower than in tomato, while only 2.86% penetrated the marigold roots. None of the J2 was found in the garlic roots (Fig. 2).

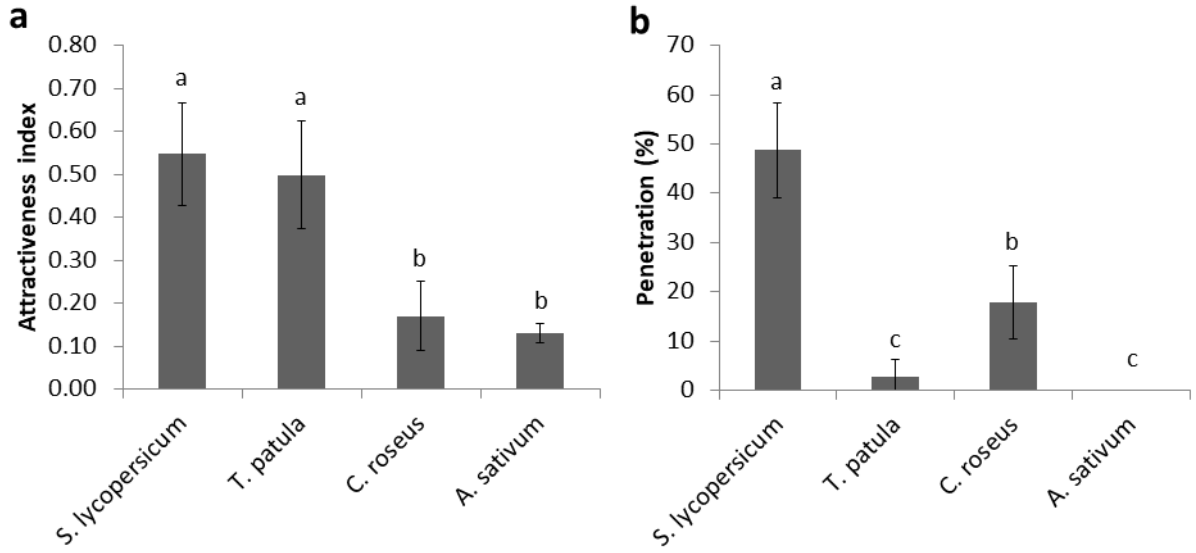

Fig. 2 Attractiveness index (**a**) and penetration (**b**) percentage of J2s of *Meloidogyne javanica* in the roots of tomato (*S. lycopersicum*), marigold (*T. patula*), Madagascar periwinkle (*C. roseus*) and garlic (*A. sativum*). Bars with the same letters do not differ significantly by Tukey's test at 5% of significance.

The development of J2s that penetrated the Madagascar periwinkle and marigold roots was affected and they failed to reach the adult stage in the evaluated period, while in the tomato roots, the J2s normally developed until reaching the adult stage (Fig. 3).

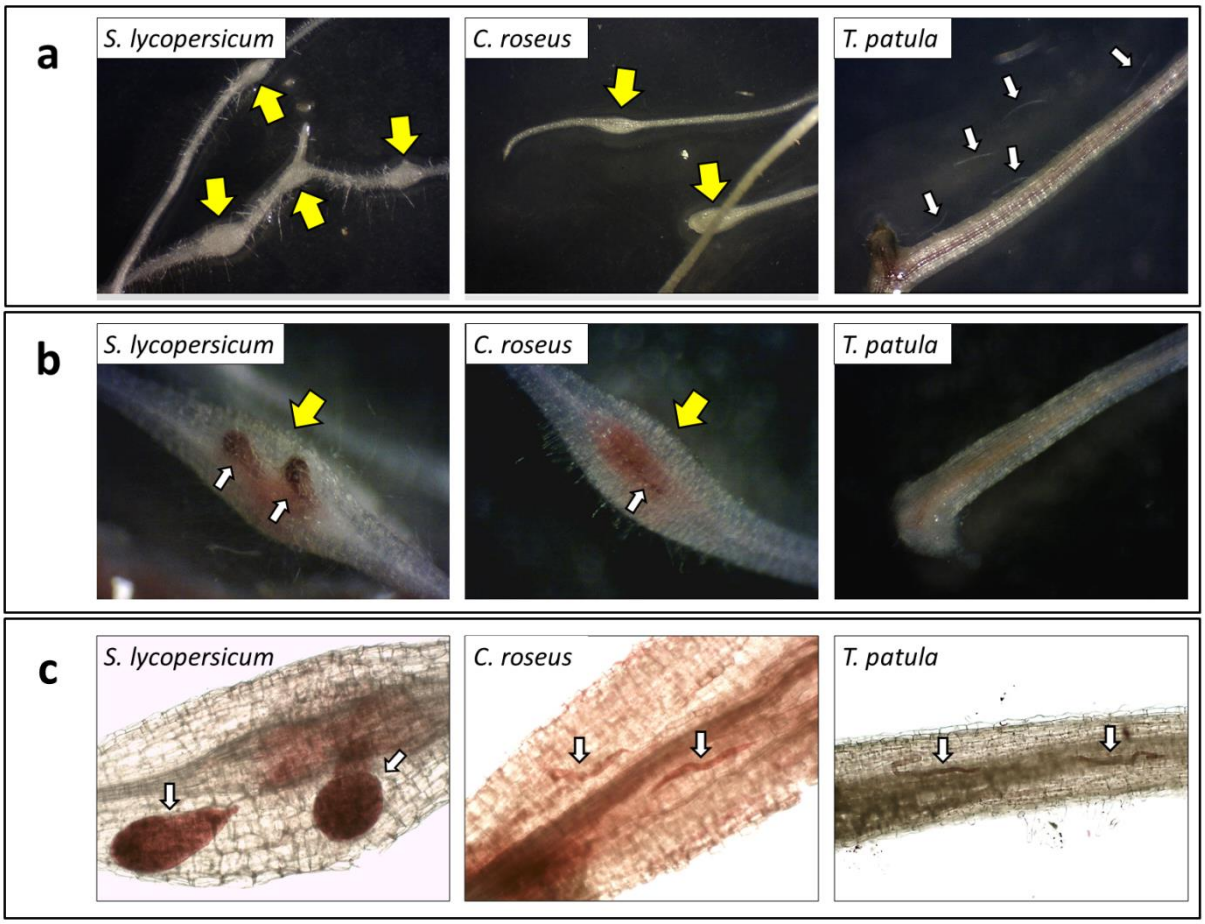

Fig. 3 Penetration of *Meloidogyne javanica* in tomato (*S. lycopersicum*), Madagascar periwinkle (*C. roseus*) and marigold (*T. patula*) roots. **a**) Roots on agar–water medium 17 days after the introduction of J2s. **b**) Roots after staining the nematodes. **c**) Root blades with stained nematodes. The yellow arrows indicate galls, and the white arrows indicate nematodes.

3.2 Chemotactic responses of *Meloidogyne javanica* to root exudates of medicinal plants

The root exudates of marigold and tomato were highly attractive to *M. javanica* since chemotaxis index (CI) values were greater than 0.2 (Wang et al. 2019). Lettuce and Madagascar periwinkle had CI values between 0.2-0.1 which are considered slightly attractive. The root exudates of garlic and yarrow did not attract the J2s by presenting CI values below 0.1 (Fig. 4).

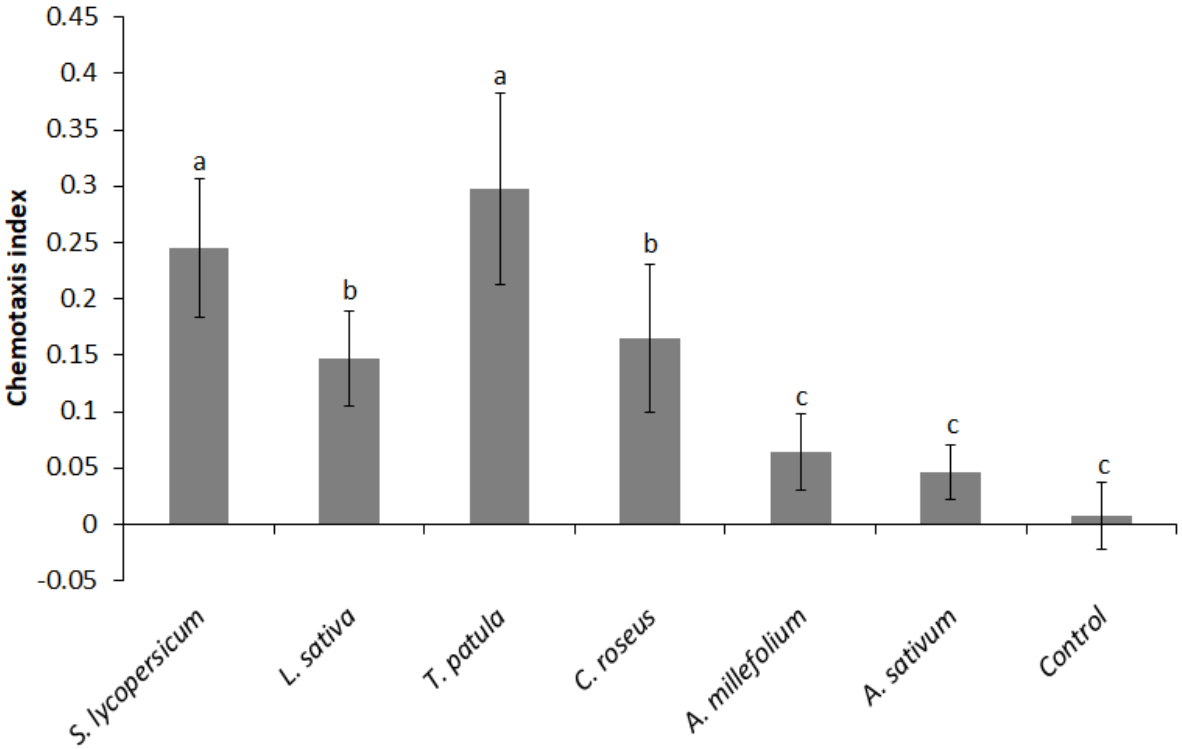

Fig. 4. Chemotactic response of *M. javanica* second-stage juveniles (J2) to tomato (*S. lycopersicum*), lettuce (*L. sativa*), marigold (*T. patula*), Madagascar periwinkle (*C. roseus*), yarrow (*A. millefolium*) and garlic (*A. sativum*) root exudates. The control was distilled water. Bars with the same letters were grouped by the Scott–Knott test at 5% of significance.

3.3 Pot intercropping of tomato with medicinal plants in *Meloidogyne javanica* infested substrate

There was a significant reduction ($p < 0.001$) in the total number of *M. javanica* eggs by intercropping the tomato with medicinal plants compared to the control (two tomato plants per pot). By evaluating only the tomato plant in each treatment, the number of eggs was similar ($p = 0.3189$). The number of eggs was smaller in marigold and a higher number of eggs was observed in yarrow roots compared to control ($p < 0.001$) (Table 1).

Table 1. Number of *Meloidogyne javanica* eggs on the root system of tomato and medicinal plants.

Intercrops*	Total number of eggs per pot (±SE**)	Number of eggs/g of root (±SE)	
		Tomato plants	Medicinal plants
Control with tomato	239,867 (±11,416) a***	7,199 (±1,137) a	-
Marigold	123,015 (±19,976) b	5,556 (±892) a	111 (±38) c
Madagascar periwinkle	154,982 (±16,026) b	4,894 (±797) a	249 (±46) b
Yarrow	132,884 (±12,795) b	4,480 (±373) a	772 (±30) a
Garlic	166,750 (±10,359) b	5,189 (±789) a	*NA*****

*Plant intercropped wot tomato, where the control consists of two tomato plants per pot, instead of tomato with the medicinal plant. **SE = standard error. ***Means with the same letters in the columns do not differ significantly by Tukey's test at 5% of significance. ****NA: not assessed.

Only the garlic promoted root fresh mass higher than the control for the tomato plant ($p = 0.0308$). In the treatments in which tomato plants were intercropped with Madagascar periwinkle, yarrow and garlic there were higher stem diameters and heights ($p < 0.001$) compared to the control (Table 2).

Table 2. Tomato and medicinal plants growth under the intercropping system.

Intercrops*	Tomato plants		
	Root fresh mass (g) (±SE**)	Stem diameter (cm) (±SE)	Height (cm) (±SE)
Control with tomato	19.30 (±2.15) b***	1.00 (±0.04) c	149.85 (±3.80) c
Marigold	23.40 (±3.63) ab	1.06 (±0.03) bc	153.10 (±7.17) bc
Madagascar periwinkle	33.20 (±3.06) ab	1.24 (±0.01) a	183.70 (±1.69) a
Yarrow	29.40 (±1.82) ab	1.22 (±0.02) a	172.40 (±4.68) ab
Garlic	36.00 (±5.16) a	1.18 (±0.03) ab	182.70 (±2.58) a

*Plant intercropped wot tomato, where the control consists of two tomato plants per pot, instead of tomato with the medicinal plant. **SE = standard error. ***Means with the same letters in the columns do not differ significantly by Tukey's test at 5% of significance.

3.4 Pot intercropping of lettuce with medicinal plants in *Meloidogyne javanica* infested substrate

There was a significant reduction ($p < 0.001$) in the total number of *M. javanica* eggs per pot in the treatments cultivating lettuce in an intercropped system with medicinal plants when compared to the control (two lettuce plants per pot). When evaluating only the lettuce

plants in each treatment, there was a significant difference ($p = 0.0054$) between the treatments in the number of eggs, where the intercropping with Madagascar periwinkle presented a lower number of eggs than the control (two lettuce plants per pot). For the medicinal plants, there was no significant difference in the number of eggs per gram of root ($p = 0.5815$) (Table 3).

Table 3. Number of *M. javanica* eggs on the root system of lettuce and medicinal plants.

Intercrops*	**Total number of eggs per pot (±SE**)**	**Number of eggs/g of root (±SE)**	
		Lettuce plants	**Medicinal plants**
Control with lettuce	5576 (±291) a***	65 (±6) a	-
Marigold	1883 (±347) b	40 (±9) ab	5 (±1) a
Madagascar periwinkle	2076 (±288) b	21 (±3) b	10 (±2) a
Yarrow	2920 (±497) b	41 (±6) ab	11 (±2) a
Garlic	2696 (±453) b	37 (±7) ab	9 (±3) a

*Plant intercropped wot tomato, where the control consists of two tomato plants per pot, instead of tomato with the medicinal plant. **SE = standard error. ***Means with the same letters in the columns do not differ significantly by Tukey's test at 5% of significance.

Regarding the effects of the treatments on the growth of lettuce plants, only in the intercropping systems of lettuce with Madagascar periwinkle or garlic was observed significant ($p < 0.001$) higher lettuce root fresh mass compared to the control (two lettuce plants per pot). In the intercropping system of lettuce with Madagascar periwinkle, yarrow or garlic, lettuce shoot fresh mass was significant ($p <0.001$) higher when compared to the control with two lettuce plants (Table 4).

Table 4. Lettuce and medicinal plants growth under the intercropping system.

Intercrops*	Lettuce plants	
	Root fresh mass (g) (±SE**)	Shoot fresh mass (g) (±SE)
Control with lettuce	47.40 (±2.74) b***	298.80 (±18.07) b
Marigold	45.00 (±1.36) b	294.60 (±25.96) b
Madagascar periwinkle	95.20 (±9.47) a	585.00 (±8.23) a
Yarrow	71.20 (±8.58) ab	568.20 (±17.26) a
Garlic	73.00 (±4.50) a	538.20 (±15.42) a

*Plant intercropped wot tomato, where the control consists of two tomato plants per pot, instead of tomato with the medicinal plant. **SE = standard error. ***Means with the same letters in the columns do not differ significantly by Tukey's test at 5% of significance. The root fresh mass from lettuce plants was transformed by log(x) before analysis.

4. Discussion

The estimation of root attraction, root exudates chemotaxis, J2 penetration and verification of the *M. javanica* development in this work improved the knowledge of how medicinal plants act in RKN management in lettuce and tomato plants. For instance, marigold and Madagascar periwinkle attracted *M. javanica* J2s, but once inside the roots they failed to complete their cycle. In addition, pot intercropping made it possible to accurately assess the RKN populations, proving the efficacy of medicinal plants intercropped with lettuce or tomato crops in reducing *M. javanica* population.

Root exudates play an important role in the interaction of plants with the rhizosphere microbiota and plant defense (Dennis et al. 2010; Baetz and Martinoia 2014; Homulle et al. 2022). Plant-parasitic nematodes are typically part of the soil ecosystem and exhibit chemotactic responses to host or non-host roots (Haichar et al. 2014; Wang et al. 2021). In this study, the J2s of *M. javanica* responded differently to the root exudates of the six species evaluated. The *M. javanica* J2s were attracted by the root exudates of tomato and lettuce, which are susceptible cultivars. The J2s were also attracted by the root exudates of marigold (*Tagetes patula*) and Madagascar periwinkle (*Catharanthus roseus*), while they showed a random response when subjected to root exudates of garlic (*Allium sativum*) and yarrow (*Achillea millefolium*) (Fig. 4). These results support that a plant being susceptible or resistant

386 to *M. javanica* is not only determined by the response of J2s attraction (Linsell et al. 2014).
387 Non-host plants release root exudates containing toxic substances in the soil that act in
388 various ways in the control of RKN, suppressing egg hatching, affecting the growth of
389 juveniles, affecting the nervous system of juveniles and blocking the attraction of juveniles
390 that are in the soil looking for a host (Torto et al. 2018a; Sikder and Vestergård 2020).

391 Root exudates of marigold are reported as a repellent to J2s of *Meloidogyne incognita*
392 (Shivakumara et al. 2018), while in the present study, marigold was highly attractive to *M.*
393 *javanica* (Fig. 4). Different species of RKN may respond differently to the same plant
394 exudates, which reinforce the importance to verify each situation to avoid misguided
395 recommendation (Sikder and Vestergård 2020). This divergent results in marigold may also
396 be related to the concentration of root exudates, which may influence the response of J2s
397 (Kirwa et al. 2018; Shivakumara et al. 2018; Zhai et al. 2018).

398 Besides the attractive stimulus of marigold and Madagascar periwinkle roots for *M.*
399 *javanica* J2s, was observed: 1) a reduction in the penetration of the J2s into their roots and 2)
400 failure in the development of the J2s that penetrated the roots, avoiding their development
401 and, consequently, the adult reproduction. These resistance mechanisms have been previously
402 reported in different crops resistant to *Meloidogyne* spp. (Herman et al. 1991; Khan and Khan
403 1991; Windham and Williams 1994; Anwar and Mckenry 2000; Linsell et al. 2014). Marigold
404 roots were previously demonstrated to be attractive to other RKN (Nježić et al. 2014; Oliveira
405 et al. 2020). However, to our knowledge, no studies have investigated the penetration and
406 development of *Meloidogyne* spp. in Madagascar periwinkle roots. A previous study verified
407 the host suitability of Madagascar periwinkle for *M. paranaensis*, indicating a high rate of
408 galls and low reproductive factor; thus, Madagascar periwinkle was considered a non-host
409 plant for this nematode (de Mendonça et al. 2017). The results obtained in this study confirm
410 the attractiveness of Madagascar periwinkle (Fig. 4) and demonstrate its effect of disturbing

411 the development of *M. javanica* juveniles that penetrated the roots (Fig. 3). These resistance
412 mechanisms are important mechanisms of action in the management of *Meloidogyne* spp.
413 when performing the intercropping systems with medicinal plants.

414 Marigold (*Tagetes* spp.) is already applied and widely used in the management of
415 RKN by intercropping, and many studies have shown efficient results, with a reduction in the
416 population of *Meloidogyne* spp. (El-Haddad et al. 2003; El-Hamawi et al. 2004; Verma 2006;
417 Kamunya et al. 2008; Tringovska et al. 2015; Xie et al. 2016). The results of the treatment in
418 which marigold (*T. patula*) was cultivated with lettuce leading to a reduction in the total
419 number of *M. javanica* eggs per pot (Table 3), add to the existing knowledge on this topic.
420 However, there are no studies investigating the cultivation of Madagascar periwinkle or
421 yarrow intercropped with other crops to assist in the management of RKN. Thus, for the first
422 time, a reduction in *M. javanica* population (represented by the total number of eggs per pot)
423 was observed through the cultivation of Madagascar periwinkle or yarrow in a pot
424 intercropping system with tomato, and lettuce. When cultivating garlic in an intercropping
425 system with tomato or lettuce, there was a reduction in the final population of *M. javanica*.
426 Garlic and other species of the genus *Allium* were able to reduce *Meloidogyne* spp. population
427 in different crops and promote plant growth by intercropping systems (Abdel-Baset and Allah
428 2020; Seman et al. 2020; Nie et al. 2021; Detrey et al. 2022). These results indicate that
429 *Allium* spp. have the potential to be used in intercropping systems for the management of
430 root-knot nematodes and to improve plant growth.

431 The cultivation of medicinal plants in the management of RKN can provide benefits,
432 improving plant growth and increasing yield (Tringovska et al. 2015). For the success of the
433 system, it is important to study the effects of each intercrop on the main crop production.
434 Garlic, Madagascar periwinkle and yarrow favored the development of both lettuce and
435 tomato. Those medicinal plants not only reduced the RKN population but also improved

tomato and lettuce growth. Medicinal plants are reported to be a profitable option in intercropping systems by promoting other crops' growth (Dai et al. 2013; Zhang et al. 2020). The Brazilian market for medicinal plants was estimated at approximately US $400 million per year, with annual growth of approximately 12% (Carvalho et al. 2018), while the global market was estimated at approximately US $62 billion per year, with annual growth of approximately 10 to 20% (Alamgir 2017). Therefore, performing diversified planting by adding medicinal plants to assist in the management of RKN makes it possible to maintain some production of traditional crops while increasing income by cultivating medicinal plants between crop rows. However, before field tests, laboratory evaluations of the attraction of roots and their exudates, J2 penetration and development inside the roots, as well as pot intercropping studies can give more insights into the effects of field associating plants on RKN populations and plant growth.

5. Conclusion

Attractive medicinal plants (marigold and Madagascar periwinkle) avoid the penetration of *M. javanica* J2 in their roots, and the majority of the J2 which penetrated could not complete their cycle. Madagascar periwinkle, yarrow and garlic improve tomato and lettuce shoot growth, being promising medicinal plants to be intercropped with tomato and lettuce, in addition to the management of *M. javanica*.

Supplementary Materials

Fig. S1. Chemotaxis assay to observe the effects of root exudates on the movement of *M. javanica*. The J2s were placed in the center (neutral area) of a 9 cm diameter Petri dish, the root exudates were added to the test area (+), and distilled water was added to the control area (-). Adapted from Wang et al. (2019).

Fig. S2. Second-stage juveniles (J2s) of *Meloidogyne javanica* around the roots of tomato (*S. lycopersicum*) (**a**), marigold (*T. patula*) (**b**), Madagascar periwinkle (*C. roseus*) (**c**) and garlic (*A. sativum*) (**d**). The white arrows indicate the nematodes.

Author Contributions

V.P.C.: investigation, formal analysis, visualization, writing – original draft. W.C.T.: formal analysis, visualization, writing – original draft, writing – review & editing. F.A.R.: visualization, writing – review & editing, supervision. A.J.M.O.: formal analysis, visualization, writing – original draft. K.M.F.F.: investigation, writing – original draft. B.M.S.: investigation, writing – original draft. V.P.C.: resources, writing – review & editing. J.C.P.S.: conceptualization,visualization,writing – review & editing.F.H.V.M.: conceptualization, writing – review & editing, supervision. J.D.: conceptualization, writing – review & editing, supervision.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. The authors thank the CAPES, FAPEMIG and CNPq institutions for granting scholarships and for supporting research in our institution. They also thank the Laboratory of Nematology of the Plant Pathology Department (DFP) and the Plant Tissue Culture Laboratory of the Department of Agriculture (DAG) of the Federal University of Lavras (Universidade Federal de Lavras - UFLA) for the structure provided for the experiments and for the support of the technicians and teachers. The authors thank the Clerio Rodrigues Ribeiro, Sansão Augusto Germano, RegianeAlvesBelizario and Maria LuizaPaiva de Oliveira for assisting in the laboratory activities.

Conflicts of Interest

The authors declare no conflict of interest.

References

A. Hussein R, A. El-Anssary A (2019) Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. In: Herbal Medicine. IntechOpen

Abdel-Baset SH, Allah AMA (2020) Effect of Intercropping Onion with Sugar Beet on Productivity of Both Crops and Root-Knot Nematodes Control under Different Onion Plant Densities and Slow-Release N Fertilizer Rates. J Plant Prod Sci 9:61–75. https://doi.org/10.21608/jpps.2020.157286

Alamgir ANM (2017) Cultivation of Herbal Drugs, Biotechnology, and In Vitro Production of Secondary Metabolites, High-Value Medicinal Plants, Herbal Wealth, and Herbal Trade. In: Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1. Springer International Publishing, pp 379–452

Ali JG, Alborn HT, Stelinski LL (2011) Constitutive and induced subterranean plant volatiles attract both entomopathogenic and plant parasitic nematodes. J Ecol 99:26–35. https://doi.org/10.1111/j.1365-2745.2010.01758.x

Anwar SA, Mckenry M V (2000) Penetration, development and reproduction of Meloidogyne arenaria on two new resistant Vitis spp. Nematropica 30:9–18

Baetz U, Martinoia E (2014) Root exudates: The hidden part of plant defense. Trends Plant Sci 19:90–98. https://doi.org/10.1016/j.tplants.2013.11.006

Carvalho ACB, Lana TN, Perfeito JPS, Silveira D (2018) The Brazilian market of herbal medicinal products and the impacts of the new legislation on traditional medicines. J Ethnopharmacol 212:29–35. https://doi.org/10.1016/j.jep.2017.09.040

Čepulyte R, Danquah WB, Bruening G, Williamson VM (2018) Potent Attractant for Root-Knot Nematodes in Exudates from Seedling Root Tips of Two Host Species. Sci Rep 8:. https://doi.org/10.1038/s41598-018-29165-4

Collange B, Navarrete M, Peyre G, et al (2011) Root-knot nematode (Meloidogyne) management in vegetable crop production: The challenge of an agronomic system analysis. Crop Prot 30:1251–1262. https://doi.org/10.1016/j.cropro.2011.04.016

D'Addabbo T, Argentieri MP, Radicci V, et al (2017) Artemisia annua compounds have potential to manage root-knot and potato cyst nematodes. Ind Crops Prod 108:195–200. https://doi.org/10.1016/j.indcrop.2017.06.025

Dai CC, Chen Y, Wang XX, Li PD (2013) Effects of intercropping of peanut with the medicinal plant Atractylodes lancea on soil microecology and peanut yield in subtropical China. Agrofor Syst 87:417–426. https://doi.org/10.1007/s10457-012-9563-z

de Mendonça IC, de Abreu Mattos JK, Carneiro RMDG (2017) Hospedabilidade de plantas medicinais a Meloidogyne paranaensis. Nematropica 47:49–54

Dennis PG, Miller AJ, Hirsch PR (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol Ecol 72:313–327. https://doi.org/10.1111/j.1574-6941.2010.00860.x

Detrey J, Cognard V, Djian-Caporalino C, et al (2022) Growth and root-knot nematode infection of tomato are influenced by mycorrhizal fungi and earthworms in an intercropping cultivation system with leeks. Appl Soil Ecol 169:104181. https://doi.org/10.1016/j.apsoil.2021.104181

Dong L, Li X, Huang L, et al (2014) Lauric acid in crown daisy root exudate potently regulates root-knot nematode chemotaxis and disrupts Mi-flp-18 expression to block

533 infection. J Exp Bot 65:131–141. https://doi.org/10.1093/jxb/ert356
534 Eder R, Consoli E, Krauss J, Dahlin P (2021) Polysulfides Applied as Formulated Garlic
535 Extract to Protect Tomato Plants against the Root-Knot Nematode Meloidogyne
536 incognita. Plants 10:394. https://doi.org/10.3390/plants10020394
537 El-Haddad SA, Omar MNA, El-Kattan MH (2003) Comparative studies on some components
538 of integrated management on soil borne plant pathogens affecting cucumber, grown
539 under protected agriculture. Acta Hortic. 608:219–226
540 El-Hamawi MH, Youssef MMA, Zawam HS (2004) Management of Meloidogyne incognita,
541 the root-knot nematode, on soybean as affected by marigold and sea ambrolia (damsisa)
542 plants. J Pest Sci (2004) 77:95–98. https://doi.org/10.1007/s10340-003-0034-1
543 Filgueiras CC, Willett DS, Pereira RV, et al (2016) Eliciting maize defense pathways
544 aboveground attracts belowground biocontrol agents. Sci Rep 6:1–9.
545 https://doi.org/10.1038/srep36484
546 Haichar F el Z, Santaella C, Heulin T, Achouak W (2014) Root exudates mediated
547 interactions belowground. Soil Biol Biochem 77:69–80.
548 https://doi.org/10.1016/j.soilbio.2014.06.017
549 Herman M, Hussey RS, Boerma HR (1991) Penetration and Development of Meloidogyne
550 incognita on Roots of Resistant Soybean Genotypes. J Nematol 23:155–161
551 Homulle Z, George TS, Karley AJ (2022) Root traits with team benefits: understanding
552 belowground interactions in intercropping systems. Plant Soil 471:1–26.
553 https://doi.org/10.1007/s11104-021-05165-8
554 Hussey RS, Barker KR (1973) A comparison of methods for colecting inocula of
555 meloidogyne spp including a new technique. Plant Dis Report 57:1025–1028
556 Jardim IN, Oliveira DF, Campos VP, et al (2020) Garlic essential oil reduces the population
557 of Meloidogyne incognita in tomato plants. Eur J Plant Pathol 157:197–209.
558 https://doi.org/10.1007/s10658-020-02000-1
559 Kamunya SM, Wachira FN, Lang'at J, et al (2008) Integrated management of root knot
560 nematode (Meloidogyne spp.) in tea (Camellia sinensis) in Kenya. Int J Pest Manag
561 54:129–136. https://doi.org/10.1080/09670870701757896
562 Khan AA, Khan MW (1991) Penetration and development of Meloidogyne incognita race 1
563 and Meloidogyne javanica in susceptible and resistant vegetables. Nematropica 21:71–77
564 Kirwa HK, Murungi LK, Beck JJ, Torto B (2018) Elicitation of Differential Responses in the
565 Root-Knot Nematode Meloidogyne incognita to Tomato Root Exudate Cytokinin,
566 Flavonoids, and Alkaloids. J Agric Food Chem 66:11291–11300.
567 https://doi.org/10.1021/acs.jafc.8b05101
568 Kouamé AP (2021) Effectiveness of Garlic and Onion Aqueous Extracts on Tomato Root-
569 Knot Nematodes (Meloidogyne sp.) in the Autonomous District of Yamoussoukro in
570 Central Côte d'Ivoire. Indian J Pure Appl Biosci 9:24–35. https://doi.org/10.18782/2582-
571 2845.8532
572 Linsell KJ, Riley IT, Davies KA, Oldach KH (2014) Characterization of resistance to
573 pratylenchus thornei (Nematoda) in wheat (Triticum aestivum): Attraction, penetration,
574 motility, and reproduction. Phytopathology 104:174–187.
575 https://doi.org/10.1094/PHYTO-12-12-0345-R
576 Liu W, Jones AL, Gosse HN, et al (2019) Validation of the Chemotaxis of Plant Parasitic
577 Nematodes Toward Host Root Exudates. J Nematol 51:1–10.
578 https://doi.org/10.21307/jofnem-2019-063
579 Long F, Lin YM, Hong T, et al (2019) Soil sickness in horticulture and forestry: A review.
580 Allelopath J 47:57–72. https://doi.org/10.26651/allelo.j/2019-47-1-1219
581 Maciel SL, Ferraz LCCB (1996) REPRODUÇÃO DE Meloidogyne incognita RAÇA 2 E DE
582 Meloidogyne javanica EM OITO ESPÉCIES DE PLANTAS MEDICINAIS. Sci Agric

583 53:232–236. https://doi.org/10.1590/S0103-90161996000200007
584 Maitra S, Palai JB, Manasa P, Kumar DP (2019) Potential of Intercropping System in
585 Sustaining Crop Productivity. Int J Agric Environ Biotechnol 12:39–45.
586 https://doi.org/10.30954/0974-1712.03.2019.7
587 Marques ML da S, Pimentel JP, Tavares OCH, et al (2012) Host suitability of different plant
588 species to Meloidogyne enterolobii in the state of Rio de Janeiro. Nematropica 42:304–
589 313
590 Mateus MAF, Faria CMDR, Botelho R V, et al (2014) Extratos aquosos de plantas medicinais
591 no controle de Meloidogyne incognita (Kofoid e White, 1919) Chitwood, 1949. Biosci J
592 30:730–736
593 McSorley R, McGovern RJ (2000) Effects of Solarization and Ammonium Amendments on
594 Plant-Parasitic Nematodes. J Nematol 32:537–541
595 Mendoza-De Gives P (2022) Soil-Borne Nematodes: Impact in Agriculture and Livestock and
596 Sustainable Strategies of Prevention and Control with Special Reference to the Use of
597 Nematode Natural Enemies. Pathogens 11:. https://doi.org/10.3390/pathogens11060640
598 Murungi LK, Kirwa H, Coyne D, et al (2018) Identification of Key Root Volatiles Signaling
599 Preference of Tomato over Spinach by the Root Knot Nematode Meloidogyne incognita.
600 J Agric Food Chem 66:7328–7336. https://doi.org/10.1021/acs.jafc.8b03257
601 Nie CR, Feng Y, Cheng XH, Cai ZQ (2021) Intercropping with Chinese leek decreased
602 Meloidogyne javanica population and shifted microbial community structure in Sacha
603 Inchi plantation. J Agric Sci 159:404–413. https://doi.org/10.1017/S0021859621000708
604 Nježić B, De Sutter N, Moens M (2014) Interaction of Tagetes patula cv. Single Gold with
605 the life cycle of the plant-parasitic nematodes Meloidogyne chitwoodi and Pratylenchus
606 penetrans. Russ J Nematol 22:101–108
607 Oka Y (2010) Mechanisms of nematode suppression by organic soil amendments-A review.
608 Appl Soil Ecol 44:101–115. https://doi.org/10.1016/j.apsoil.2009.11.003
609 Oliveira AK dos S, Pedrosa EMR, Dickson DW, et al (2020) Migration and penetration of
610 Meloidogyne enterolobii and M. incognita in soil columns with tomato and marigold.
611 Eur J Plant Pathol 158:591–598. https://doi.org/10.1007/s10658-019-01889-7
612 Phani V, Khan MR, Dutta TK (2021) Plant-parasitic nematodes as a potential threat to
613 protected agriculture: Current status and management options. Crop Prot 144:.
614 https://doi.org/10.1016/j.cropro.2021.105573
615 Pinheiro JB, Pereira RB, Suinaga FA (2014) Manejo de nematoides na cultura do tomate, 1st
616 edn. Embrapa Hortaliças, Brasília
617 R Core Team (2019) R: A language and environment for statistical computing. https://www.r-
618 project.org/
619 Ralmi NHAA, Khandaker MM, Mat N (2016) Occurrence and control of root knot nematode
620 in crops: A review. Aust J Crop Sci 10:1649–1654.
621 https://doi.org/10.21475/ajcs.2016.10.12.p7444
622 Ribeiro AC, Guimarães PTG, Alvarez V. VH (1999) 5ª Aproximação - Recomendações para
623 o uso de corretivos e fertilizantes em Minas Gerais. Comissão de Fertilidade do Solo do
624 Estado de Minas Gerais, Viçosa, Minas Gerais
625 Rinaldi LK, Nunes J, Montecelli TDN (2014) Efeito de texturas do solo sobre populações de
626 Meloidogyne javanica e Meloidogyne incognita em soja. Cultiv o Saber 7:83–101
627 Rocha F da S, Muniz M de FS, Campos VP (2005) Coloração de fitonematoides com corantes
628 usados na indústria alimentícia brasileira. Nematol Bras 29:293–297
629 Rodríguez-kábana R, Canullo GH (1992) Cropping systems for the management of
630 phytonematodes. Phytoparasitica 20:211–224. https://doi.org/10.1007/BF02980843
631 Salem FM, Osman GY (1988) Effectiveness of tagetes natural exudates onMeloidogyne
632 javanica (Chitwood) nematode. Anzeiger für Schädlingskd Pflanzenschutz

633 Umweltschutz 61:17–19. https://doi.org/10.1007/BF01906121
634 Seid A, Fininsa C, Mekete T, et al (2015) Tomato (Solanum lycopersicum) and root-knot
635 nematodes (Meloidogyne spp.)-a century-old battle. Nematology 17:995–1009.
636 https://doi.org/10.1163/15685411-00002935
637 Seman A, Awol S, Mashilla D (2020) Integrated management of Meloidogyne incognita in
638 tomato (Solanum lycopersicum) through botanical and intercropping. African J Agric
639 Res 15:492–501. https://doi.org/10.5897/AJAR2019.14040
640 Sgorlon LFF, Silva EHC, Soares RS, et al (2018) Host status of crispy-leaf lettuce cultivars to
641 root-knot nematodes. Biosci J 34:1319–1325. https://doi.org/10.14393/BJ-v34n5a2018-
642 39387
643 Shivakumara TN, Dutta TK, Rao U (2018) A novel in vitro chemotaxis bioassay to assess the
644 response of Meloidogyne incognita towards various test compounds. J Nematol 50:487–
645 494. https://doi.org/10.21307/jofnem-2018-047
646 Sikder M, Vestergård M (2020) Impacts of Root Metabolites on Soil Nematodes. Front Plant
647 Sci 10:1–18. https://doi.org/10.3389/fpls.2019.01792
648 Sikora RA, Fernandez E (2005) Nematode parasites of vegetables. In: Luc M, Sikora RA,
649 Bridge J (eds) Plant-parasitic nematodes in subtropical and tropical agriculture, 2nd edn.
650 CAB International, Wallingford, UK, pp 319–391
651 Silva M de F, Paulo Campos V, Barros AF, et al (2020) Medicinal plant volatiles applied
652 against the root-knot nematode Meloidogyne incognita. Crop Prot 130:105057.
653 https://doi.org/10.1016/j.cropro.2019.105057
654 Singh S, Singh B, Singh AP (2015) Nematodes: A Threat to Sustainability of Agriculture.
655 Procedia Environ Sci 29:215–216. https://doi.org/10.1016/j.proenv.2015.07.270
656 Sivasubramaniam N, Hariharan G, Zakeel MCM (2020) Sustainable Management of Plant-
657 Parasitic Nematodes: An Overview from Conventional Practices to Modern Techniques.
658 In: Ansari RA, Rizvi R, Mahmood I (eds) Management of Phytonematodes: Recent
659 Advances and Future Challenges. Springer, Singapore, pp 353–399
660 Torto B, Cortada L, Murungi LK, et al (2018a) Management of Cyst and Root Knot
661 Nematodes: A Chemical Ecology Perspective. J Agric Food Chem 66:8672–8678.
662 https://doi.org/10.1021/acs.jafc.8b01940
663 Torto B, Kirwa H, Kihika R, Murungi LK (2018b) Strategies for the Manipulation of Root
664 Knot Nematode Behavior with Natural Products in Small Scale Farming Systems. ACS
665 Symp Ser 1294:114–126. https://doi.org/10.1021/bk-2018-1294.ch009
666 Tringovska I, Yankova V, Markova D, Mihov M (2015) Effect of companion plants on
667 tomato greenhouse production. Sci Hortic (Amsterdam) 186:31–37.
668 https://doi.org/10.1016/j.scienta.2015.02.016
669 Verhoeven KJF, Biere A, Harvey JA, Van Der Putten WH (2009) Plant invaders and their
670 novel natural enemies: Who is naïve? Ecol Lett 12:107–117.
671 https://doi.org/10.1111/j.1461-0248.2008.01248.x
672 Verma KK (2006) Management of root-knot nematode (Meloidogyne javanica) in fieldpea
673 (Pisum sativum) by intercropping with marigold (Tagetes erecta). Ann Agri Bio Res
674 11:121–122
675 Wang C, Masler EP, Rogers ST (2018) Responses of Heterodera glycines and Meloidogyne
676 incognita Infective Juveniles to Root Tissues, Root Exudates, and Root Extracts from
677 Three Plant Species . Plant Dis 102:1733–1740. https://doi.org/10.1094/pdis-09-17-
678 1445-re
679 Wang J, Ding Z, Bian J, et al (2021) Chemotaxis response of Meloidogyne incognita to
680 volatiles and organic acids from root exudates. Rhizosphere 17:100320.
681 https://doi.org/10.1016/j.rhisph.2021.100320
682 Wang P, Sun Y, Yang L, et al (2019) Chemotactic responses of the root-knot nematode

683 Meloidogyne incognita to Streptomyces plicatus. FEMS Microbiol Lett 366:1–7.
684 https://doi.org/10.1093/femsle/fnz234
685 Wilschut RA, Silva JCP, Garbeva P, Putten WH Van Der (2017) Belowground Plant–
686 Herbivore Interactions Vary among Plant Species with Different Degrees of Novel
687 Chemistry. Front Plant Sci 8:1–10. https://doi.org/10.3389/fpls.2017.01861
688 Windham GL, Williams WP (1994) Penetration and development of Meloidogyne incognita
689 in roots of resistant and susceptible corn genotypes. J Nematol 26:80–85
690 Xie G, Cui H, Dong Y, et al (2016) Crop rotation and intercropping with marigold are
691 effective for root-knot nematode (Meloidogyne sp.) control in angelica (Angelica
692 sinensis) cultivation. Can J Plant Sci CJPS-2016-0071. https://doi.org/10.1139/CJPS-
693 2016-0071
694 Zhai Y, Shao Z, Cai M, et al (2018) Multiple Modes of Nematode Control by Volatiles of
695 Pseudomonas putida 1A00316 from Antarctic Soil against Meloidogyne incognita. Front
696 Microbiol 9:. https://doi.org/10.3389/fmicb.2018.00253
697 Zhang X, Gao G, Wu Z, et al (2020) Responses of soil nutrients and microbial communities
698 to intercropping medicinal plants in moso bamboo plantations in subtropical China.
699 Environ Sci Pollut Res 27:2301–2310. https://doi.org/10.1007/s11356-019-06750-2
700 Zhou X, Yu G, Wu F (2011) Effects of intercropping cucumber with onion or garlic on soil
701 enzyme activities, microbial communities and cucumber yield. Eur J Soil Biol 47:279–
702 287. https://doi.org/10.1016/j.ejsobi.2011.07.001

Supplementary Material

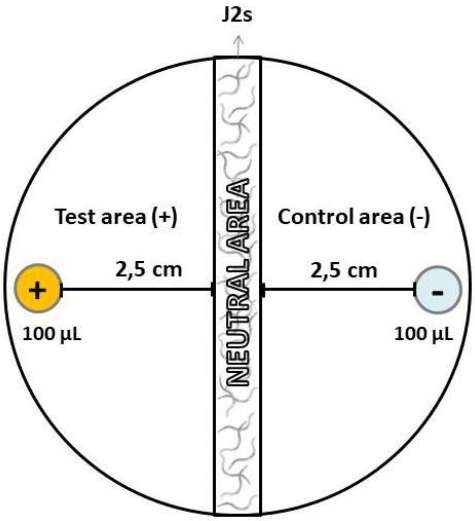

Fig. S1. Chemotaxis assay to observe the effects of root exudates on the movement of *M. javanica*. The J2s were placed in the center (neutral area) of a 9 cm diameter Petri dish, the root exudates were added to the test area (+), and distilled water was added to the control area (-). Adapted from Wang et al. (2019).

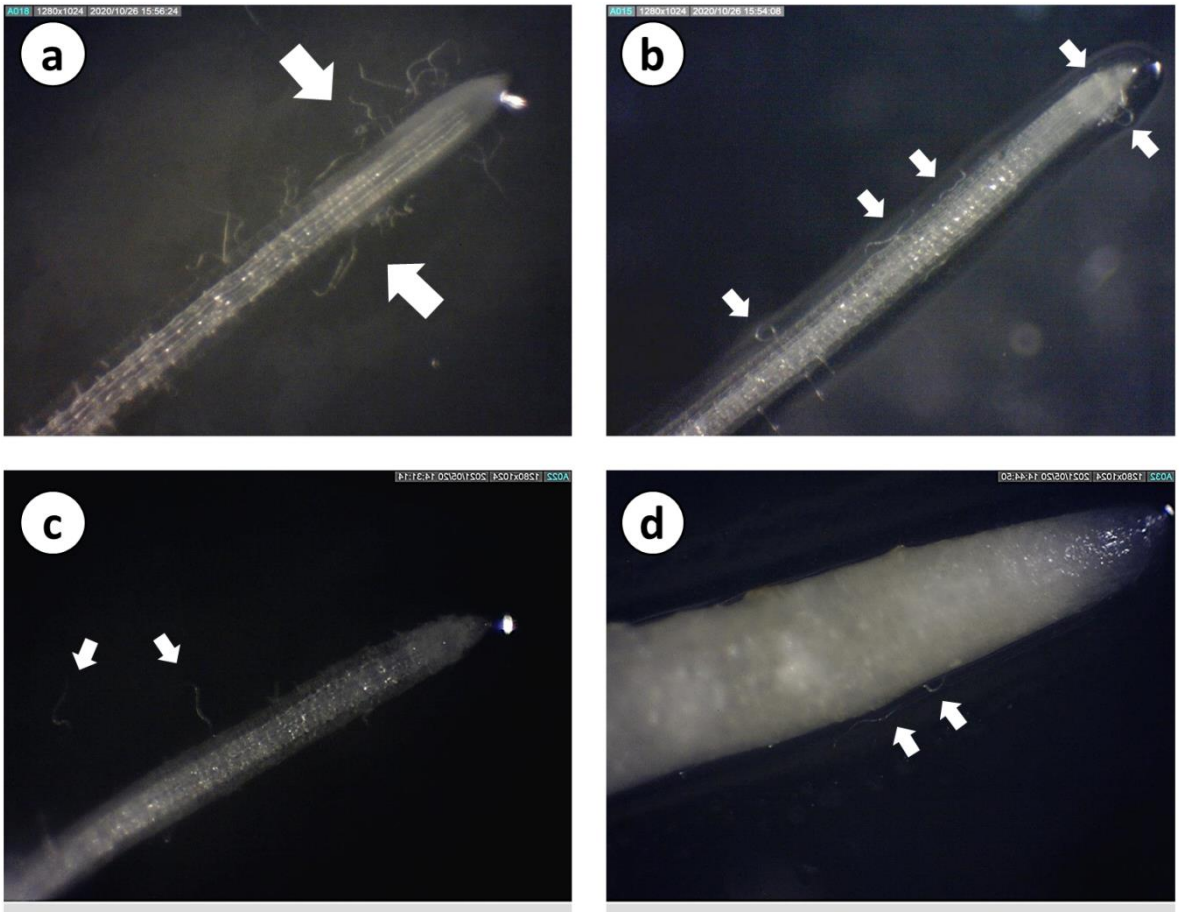

Fig. S2. Second-stage juveniles (J2s) of *Meloidogyne javanica* around the roots of **a**) tomato (*S. lycopersicum*), **b**) marigold (*T. patula*), **c**) Madagascar periwinkle (*C. roseus*) and **d**) garlic (*A. sativum*). The white arrows indicate the nematodes.

Versão preliminar submetida ao periódico: Agricultural Systems

Integrated root-knot nematode management on lettuce crops using cultural and biological practices: garlic cultivation and *Bacillus subtilis* BV09 application

ABSTRACT

CONTEXT

Root-knot nematode (RKN, *Meloidogyne* spp.) is a plant-parasitic nematode economically important for several horticultural crops, such as lettuce. RKN management is hard-working and expensive, making it necessary to take ecosystem balance into account to maintain RKN population densities below damaging levels.

OBJECTIVE

Evaluate the effect of garlic cultivation and *Bacillus subtilis* BV09 application in the integrated management of *Meloidogyne incognita* in lettuce crops.

METHODS

The experiment was carried out in a *M. incognita*-infested field, performing the treatments: lettuce monoculture (control) and garlic cultivation as a succession crop or intercropped with lettuce plants, with the application of *B. subtilis* BV09 (commercial product Biobaci®) or with no applications. During the garlic cropping cycle (146 days), three lettuce cycles were performed. The fallow soil and removal of infested roots were also carried out as cultural practices for integrated RKN management. After performing the management practices until 146 days after planting (DAP), from 147 to 271 DAP only lettuce monoculture was carried out in all plots, maintaining only the application of *B. subtilis* BV09 in the respective treatments. *M. incognita* population was evaluated before the implementation of the experiment and at each harvest. Lettuce and garlic production and income, plants' biochemical defense response and foliar nutrients content were evaluated.

RESULTS AND CONCLUSIONS

Garlic cultivation and *B. subtilis* BV09 application reduced *M. incognita* population in the field. The removal of infested roots reduced *M. incognita* population in approximately 80%. In fallow, *M. incognita* population was reduced but lower income was observed due to leaving the area unproductive for a long period. The production per garlic plant was improved by intercropping with lettuce and *B. subtilis* BV09 application, while the production per lettuce plant on its third cropping cycle was affected by the intercropping when garlic plants were large, due to competition for nutrients and solar radiation. Intercropping enabled achieving high total income from lettuce and garlic crops, as the total income obtained from lettuce monoculture. *M. incognita* attack activated plants' biochemical defense response, while the *B. subtilis* BV09 application and intercropping system helped to protect lettuce and garlic plants against the stress caused by the *M. incognita* infection.

SIGNIFICANCE

Garlic cultivation and *B. subtilis* BV09 application were efficient practices in the integrated management of *M. incognita* in lettuce crops, protecting lettuce and garlic plants against the stress caused by the *M. incognita* infection, in addition to provide income even during the RKN management.

Keywords: root-knot nematode, cropping system, intercropping, biological control, horticulture, *Allium sativum*.

1. Introduction

The occurrence of pests and diseases in agricultural fields causes losses to farmers, reducing production and increasing investment in control strategies. Plant-parasitic nematodes (PPN) have caused a high level of damage to crops (Ralmi et al., 2016; Singh et al., 2015). Root-knot nematodes (*Meloidogyne* spp.) are cosmopolitan and generalist species of PPN, being considered economically important for several crops (Sikora and Fernandez, 2005). Lettuce (*Lactuca sativa* L.), which is a crop widely consumed around the world, is a host plant and faces losses of more than 80% of its production due to root-knot nematode (RKN) attacks (Charchar and Moita, 2005).

The management of RKN is expensive and its eradication is practically impossible, making it necessary to implement measures taking ecosystem balance into account, and maintaining RKN population densities below damaging levels (Ralmi et al., 2016; Subedi et al., 2020). Although individually each management strategy has a limited effect, when performed together in an integrated management they help to achieve an efficient reduction in RKN populations in plants and agricultural soils, in addition, to help improving crop yields, favoring sustainable systems (Akhtar, 1997; Subedi et al., 2020).

Integrated management of plant-parasitic nematode is the use of two or more compatible methods concurrently by combining cultural, chemical, and biological methods (Sivasubramaniam et al., 2020). In RKN infested areas, plant species cultivated and the way they are distributed in the field must be considered when developing management strategies. Performing intercropping, succession or rotating crops as cultural methods using non-host species causes a reduction in the number of roots that RKN is capable of parasitizing reducing its reproductive potential and, consequently, causing a reduction in RKN population in the soil (Abd-Elgawad, 2020). In addition, non-host plants can release root exudates into the soil containing toxic substances that act in different ways to control RKN, such as suppressing the hatch of second-stage juveniles (J2), affecting the growth of juveniles, reaching the nervous system of juveniles, and blocking the attraction of juveniles that are on the ground looking for a host (Torto et al., 2018). Garlic is an antagonistic plant to RKN which has nematicidal properties (Eder et al., 2021; Jardim et al., 2020; Kouamé, 2021) and was able to reduce *M. incognita* population density when intercropped with tomato plants in a pot experiment (Seman et al., 2020), but with a lack of field experiments cultivating susceptible crops intercropped with garlic to confirm its effect on RKN management in agricultural fields.

Biological control has been growing as a RKN management strategy due to environmental concerns and demands from organic agriculture, presenting less impact on the

environment than chemical nematicides (Sivasubramaniam et al., 2020). Bionematicides have a growing market worldwide, reaching around 82% of the total market for nematicides in Brazil in 2019/2020, with much of them being formulations based on *Bacillus* genus bacteria (Machado, 2022). *Bacillus subtilis* effectiveness in controlling *M. incognita* is confirmed by several studies (Adam et al., 2014; Basyony and Abo-Zaid, 2018; Cao et al., 2019; Hussain et al., 2020), acting as bionematicide repelling or killing second-stage juveniles (J2) and inhibiting egg hatching (Cao et al., 2019; Dawar et al., 2008), in addition to acting indirectly by promoting resistance induction in plants (Adam et al., 2014).

Cultural methods have effects on microbial abundance, diversity, and activity in the soil, while biological control explores the use of microbial agents, thus, it is important to study their compatibility in the management of RKN (Sivasubramaniam et al., 2020; Viaene et al., 2013). Several factors must be considered when carrying out management strategies in the field besides the effect on the RKN population, such as its positive and negative effects on the crop of interest. In this context, the objective was to evaluate the effect of garlic cultivation and *Bacillus subtilis* BV09 application in integrated of *Meloidogyne incognita* in lettuce crops, as well as its effect on lettuce and garlic production and income, plants' biochemical defense response and foliar nutrients content.

2. Methods

The experiment was carried out in a field at the Technology Development and Transfer Center (CDTT), which is an experimental area of the Federal University of Lavras (UFLA). In the beds where the experiment was carried out, the soil was naturally infested with *M. incognita* and okra (*Abelmoschus esculentus*) was cultivated in the beds for three months before the installation of the experiment.

A randomized block design containing two blocks with two replications of each treatment randomly distributed within each block was performed (Fig. 1**a**). It was planned as an incomplete factorial scheme with split plots in time, consisting of three factors: lettuce, garlic, and application of *B. subtilis* BV09 (commercial product Biobaci®) (Fig. 1**c**). The application of *B. subtilis* BV09 in the absence of lettuce and garlic (fallow soil) was not performed because it did not make sense to apply *B. subtilis* BV09 in the absence of plants.

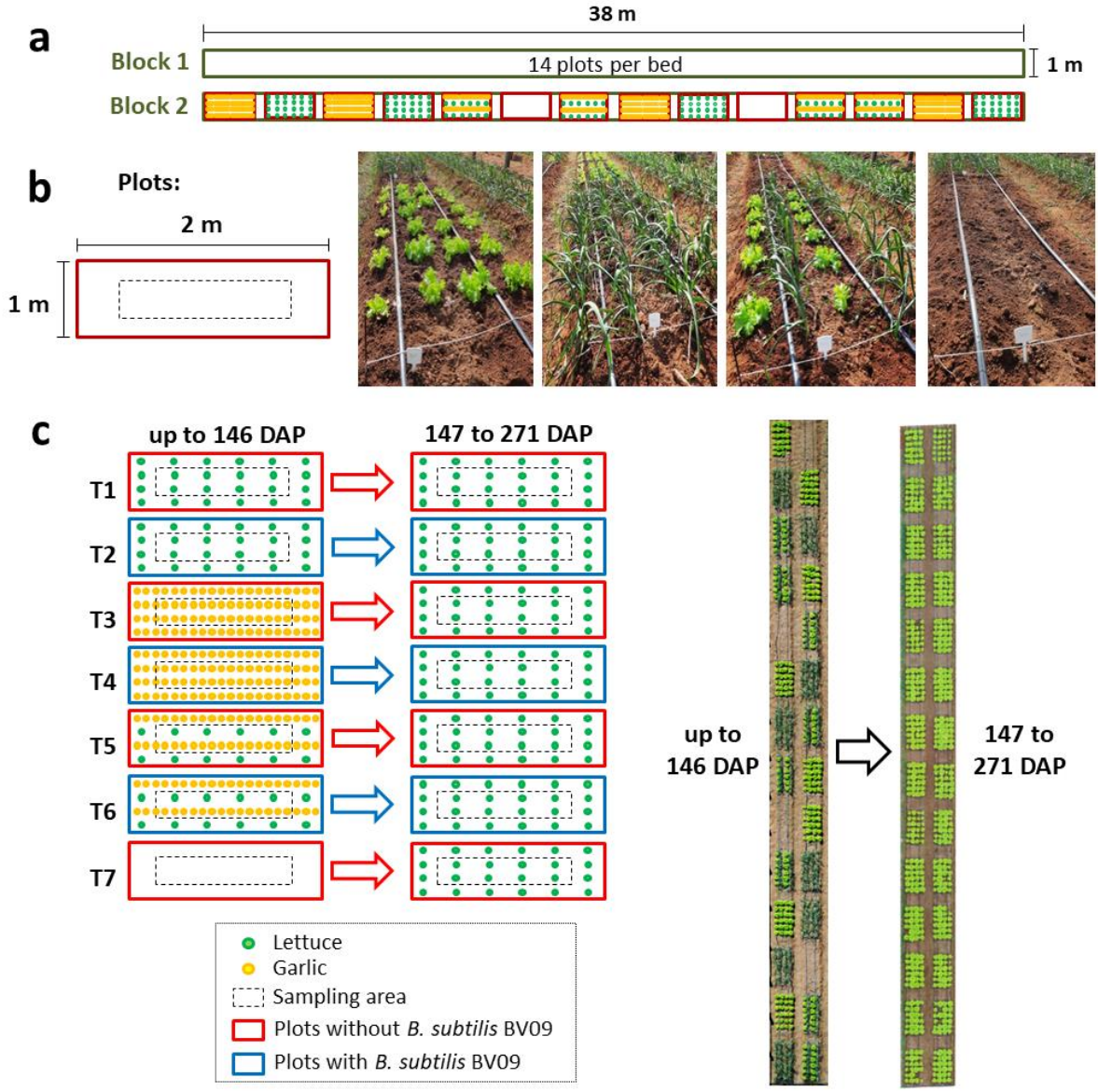

Fig. 1. Experimental design and representation of the treatments over time. **a**) **s**ize of beds showing the random distribution of plots in the blocks, **b**) size of plots and **c**) distribution of plants over time (0 to 271 DAP) according to the treatments carried out in the field. DAP: days after planting; T1: lettuce monoculture; T2: lettuce monoculture with the application of *B. subtilis* BV09; T3: garlic monoculture; T4: garlic monoculture with the application of *B. subtilis* BV09; T5: lettuce intercropped with garlic; T6: lettuce intercropped with garlic and with the application of *B. subtilis* BV09; T7: fallow soil.

During the first 146 days after planting (DAP), different *M. incognita* management strategies were performed. Up to the 146 DAP, as the lettuce plants were harvested (in the treatments T1, T2, T5, and T6) their roots were pulled out of the soil and removed from the productive area, disposing of the infested roots. From 147 to 271 DAP, lettuce monoculture was carried out in all plots, without removing the infested roots from the soil, to evaluate the

M. incognita population in lettuce crops after performing the management practices, maintaining only the application of *B. subtilis* BV09 in the respective treatments (Fig. 1**c**).

Garlic is usually planted in autumn (Takagi, 2020), thus, the experiment started in April 2021 (15/04/2021) and ended in January 2022 (11/01/2022). During this period of approximately nine months (total of 271 days), the weather conditions oscillated according to the seasons of the year (autumn, winter, spring and summer) and the local climate (Fig. S1).

2.1 Soil preparation and fertilization

The chemical characterization of the soil showed: pH = 6.6; P = 302.53 mg/dm^3; K = 210.93 mg/dm^3; Na = 0.00 mg/dm^3; Ca = 4.00 cmolc/dm^3; Mg = 1.05 cmolc/dm^3; Al = 0.10 cmolc/dm^3; H+Al = 3.10 cmolc/dm^3; SB = 5.59 cmolc/dm^3; t = 5.69 cmolc/dm^3; T = 8.69 cmolc/dm^3; Base saturation = 64.34%; Aluminum saturation = 1.76%; Organic matter = 2.28 dag/kg; P-rem = 31.80 mg/L; Zn = 20.50 mg/dm^3; Fe = 43.40 mg/dm^3; Mn = 20.90 mg/dm^3; Cu = 5.87 mg/dm^3; B = 0.11 mg/dm^3 and S = 3.20 mg/dm^3. Soil texture was determined through granulometric analysis, classifying the soil as clayey, with the following contents: clay = 47 dag/kg, silt = 16 dag/kg and sand = 37 dag/kg.

After removing the okra shoots, the roots were kept in the soil and the machine raised the beds and passed about four to five times per bed to homogenize the initial inoculum and incorporate the fertilizer. Fertilization was made before planting with single superphosphate, potassium chloride and ammonium sulfate. For garlic, two additional top dressings fertilization with ammonium sulfate and potassium chloride, and four foliar fertilizations with zinc sulfate and boric acid were carried out throughout the experiment. For the lettuce crop, in each cycle, top dressing fertilization with ammonium sulfate and potassium chloride, and a foliar fertilization with zinc sulfate and boric acid were carried out. The planting fertilization, with single superphosphate, potassium chloride and ammonium sulfate, was repeated in the implantation of the third (at 96 DAP) and fifth cycles (at 193 DAP) of the lettuce crop. From the fourth to the sixth cycles of lettuce, no top dressing fertilization was carried out, since fertilization was performed by fertigation in all plots.

2.2 Lettuce and garlic cultivation

Garlic cultivar 'Gigante da Lavínia Geraldo Braz' was obtained through vegetative propagation. The garlic cloves came from the Olericulture Sector of UFLA. Lettuce seedlings cultivar 'Solaris' were prepared in trays of 162 cells using the commercial substrate Carolina Soil® (composed of peat, vermiculite, class A agro-industrial organic waste and limestone).

Lettuce cultivar 'Solaris' was chosen because of its susceptibility to *M. incognita* (Sgorlon et al., 2018).

In each bed (38 meters long x 1 meter wide), 14 plots of 2 meters long x 1 meter wide were delimited, leaving about 50 cm of space between them. After plots demarcation, soil sampling was performed in each plot to quantify the number of J2 in 100 cm^3 of soil, i.e., initial population. In the plots where lettuce monoculture was performed (T1 and T2), lettuce seedlings were transplanted (with approximately 25 days) in a spacing of 25 cm between rows and 30 cm between plants (a total of 24 plants per plot). In the plots where garlic monoculture was performed (T3 and T4), garlic cloves were planted with a spacing of 25 cm between rows and 10 cm between plants (a total of 80 cloves per plot). In the plots where the lettuce was intercropped with garlic (T5 and T6), interspersed rows of lettuce and garlic were planted with a spacing of 25 cm between rows, using 30 cm between plants in lettuce rows and 10 cm between plants in garlic rows. In the plots where fallow was carried out (T7), the soil was kept exposed and with the same irrigation performed in the other treatments (Fig. 1).

The weeds were manually removed whenever necessary during the experiment. Applications of fungicide (Revus®, Syngenta, Monthey, Suíça) and insecticide (Decis®, Bayer, Gujarat, India) were performed when necessary, following the manufacturer's recommendations.

Each bed was irrigated by two drip lines. At 65 days after planting, the irrigation of garlic plants was cut off for two weeks, inducing water stress to avoid over budding and to stimulate the production of commercial bulbs (Macêdo et al., 2006). After garlic harvest, the curing process was carried out in the shade for approximately 30 days.

2.3 *Bacillus subtilis* BV09 application

The commercial product Biobaci® (Vittia, São Joaquim da Barra, SP, Brazil) which is a microbiological nematicide with *B. subtilis* BV09 in its formulation, was chosen because it has *M. incognita* as one of its targets. A dosage of 0.6 mL/m^2 was used. The equipment used for the application was a manual backpack sprayer, and the syrup was applied via drench in-furrow before planting (first, second, third, and sixth cycle) or around the plant's stem after planting (fourth and fifth cycle).

2.4 Reproductive factor of *Meloidogyne incognita* in lettuce and garlic plants

Lettuce and garlic seedlings with approximately four weeks of growth were planted in one liter pots filled with a commercial substrate composed of peat, vermiculite, class A agro-

industrial organic waste and limestone (Carolina Soil®, Santa Cruz do Sul, RS, Brazil). After one week, approximately 5,000 eggs were added to small holes made in the substrate around the plant. Plants were kept in a greenhouse and the irrigation was performed by drip irrigation according to the plant needs. Foliar fertilization was performed with NIPHOKAM 108 (Fênix Agro, Tietê, SP, Brazil) following the manufacturer's recommendations.

Approximately 60 days after the introduction of *M. incognita* eggs to the substrate, the number of eggs in plant's root system was evaluated. The eggs were extracted according to the technique of Hussey and Barker (1973). The eggs were counted under a stereoscopic microscope using a Peters camera, and three counts per replicate of each plant (lettuce and garlic) were performed. The reproductive factor (RF) was calculated by dividing the final population, evaluated in the root system of lettuce or garlic, by the initial population introduced in each pot (5,000 eggs).

2.5 Evaluation of *Meloidogyne incognita* population in soil and root samples

Soil samples from each experimental plot were collected on the day when the experiment was set up, just before lettuce and garlic planting. Over time, soil samples were collected in all experimental plots just before planting each new lettuce cycle. A final soil collection was performed in all experimental plots at the end of the experiment, at the time of lettuce harvest.

The *M. incognita* population in soil samples was evaluated by the method of Jenkins (1964), extracting free second-stage juveniles (J2) per 100 cm^3 of soil. The J2 were counted using a Peters chamber under an optical microscope, with at least two counts being performed per sample. The value used to estimate the number of J2 per sample was the average of the two counts.

At each lettuce or garlic harvest, the root systems were collected, washed, and cut into 5 mm pieces for egg extraction using the technique of Hussey and Barker (1973). At least two counts of the number of eggs were made per sample using a Peters chamber under an optical microscope. From the average of the two counts, the number of *M. incognita* eggs per gram of root was estimated.

To assess the gall index, a classification scale was used to estimate the level of RKN infestation in the root system (Bridge and Page, 1980).

2.6 Plant growth, production and productivity of lettuce and garlic

The evaluation of plant growth parameters was performed at the end of each cycle. The evaluation of garlic plants was performed at 146 days after planting (DAP). Lettuce plants were evaluated during six cycles of approximately 45 days. For lettuce plants, fresh mass of shoot and roots were evaluated. For garlic plants, fresh mass of bulb and roots were evaluated. The production per plant was represented by the commercial shoot fresh mass for lettuce plants and bulb fresh mass for garlic plants.

The lettuce productivity estimation was performed by multiplying the observed production per plant by an estimated number of lettuce plants per hectare. For the treatments in which the intercropping was carried out, the number of lettuce plants was half of the number used in monoculture. Therefore, for lettuce productivity calculation, the observed production per plant was multiplied by 100,000 plants in the monoculture and 5,000 plants in the intercropping.

Garlic bulbs were classified according to their diameter, as follows: refuse (<32 mm), class-3 (32 to 37 mm), class-4 (37 to 42 mm), class-5 (42 to 47 mm), class-6 (47 to 56 mm), and class-7 (>56 mm). Then, the total weight and number of bulbs were evaluated for each class. According to the spacing used in the field, were roughly calculated 300,300 plants per hectare in the monoculture and 150,150 plants in the intercropping. Thus, for garlic productivity estimation, the number of bulbs per hectare from each class was estimated and multiplied by the production per garlic plant observed in each class.

2.7 Gross and net income estimation of lettuce and garlic production

Gross income was calculated based on estimated productivity per hectare multiplied by the average selling price, from each harvest month, of lettuce (May/2021: R$4.88/Kg; July/2021: R$4.30/Kg; September/2021: R$3.83/Kg; October/2021: R$3.74/Kg; December/2021: R$6.43/Kg; January/2022: R$8.76/Kg) and garlic (using the commercial classification: R$17.00/Kg of bulbs from class-7; R$15.00/Kg of bulbs from class-5 and class-6; R$11.00/Kg of bulbs from class-3 and class-4) according to the supply center CEASAMINAS great unity Belo Horizonte, MG (information available at: http://www.ceasaminas.com.br/informacoesmercadogeral.asp). The costs of production per hectare of lettuce and garlic in monoculture were estimated at R$30,000.00 and R$80,000.00, respectively, according to producers under the 2021-2022 agriculture year. For the intercropping, half of the cost of production values from lettuce and garlic per hectare were used. Net income was calculated based on gross income minus the estimated cost production per hectare.

2.8 Antioxidant enzymes, H_2O_2 levels and lipid peroxidation

For each experimental plot, two samples were collected from the first fully expanded leaves in lettuce (in the first and third cycle before harvest) and garlic plants (in the beginning and at the end of the cycle before harvest). The samples were macerated in liquid nitrogen with Polyvinylpyrrolidone (PVP), identified and stored in 2 mL microtubes placed in an ultrafreezer at approximately -80°C until the analyses were performed. For each experimental plot, two biological replicates were used.

For the extraction of antioxidant enzymes, 200 mg of leaves fresh mass were macerated in liquid nitrogen and homogenized in 1.5 mL of extraction buffer (100 mM potassium phosphate, pH 7.8, 0.1 mM EDTA, 10 mM ascorbic acid). The homogenates were centrifuged at 13,000 *g* for 10 minutes at 4°C, collecting the supernatants for quantification of the enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Biemelt et al., 1998).

SOD was determined by the enzyme's ability to inhibit the photochemical reduction of nitrotetrazolium blue (NBT) (Giannopolitis and Ries, 1977). CAT was determined by the decrease in absorbance at 240 nm, every 15 seconds for 3 minutes, monitored by the consumption of hydrogen peroxide (H_2O_2) (Havir and McHale, 1987). APX was determined by monitoring the ascorbate oxidation, evaluating the reduction in absorbance at 290 nm, every 15 seconds for 3 minutes (Nakano and Asada, 1981).

For the extraction of reactive species, 200 mg of leaves fresh mass were macerated in liquid nitrogen and homogenized in 1.5 mL of 0,1% trichloroacetic acid (TCA). The homogenates were centrifuged at 12,000 *g* for 15 minutes at 4°C, collecting the supernatants for H_2O_2 and lipid peroxidation quantification. Lipid peroxidation was determined by malondialdehyde (MDA) quantification through its colorimetric reaction with thiobarbituric acid (TBA) at high temperature (Buege and Aust, 1978). H_2O_2 was quantified based on a standard curve ($y = 0.0073x + 0.0236$, $R^2 = 0.999$) with known concentrations of H_2O_2 (0 to 250 µM), measuring the absorbance at 390 nm (Velikova et al., 2000).

2.9 Nutritional analysis of lettuce and garlic leaves

For lettuce and garlic plants foliar analysis, samples composed of newly developed leaves were collected from four replicates per treatment. The leaves were dried in an oven with forced air flow and approximately 60°C to 65°C of temperature until reaching constant weight. Subsequently, foliar nutritional analyses of nitrogen, phosphorus, potassium, calcium,

magnesium, sulfur, boron, copper, iron, manganese and zinc were carried out according to the methods described by Malavolta, Vitti and Oliveira (1997).

2.10 Statistical analysis

Models for split plots on time using discrete-time factor and also autoregressive models were tested, but have no relevance for the analysis of the second-stage juveniles (J2) count in the soil. As plot variances were also very small ($3x10^{-9}$) we can assume that it is a factorial model for the time as well, but we choose to present the split-plot model as the plot variance is not null. An autoregressive model of the split-plot design was used for the analysis of the eggs' count in the roots, presenting block variance as 0.115, plot variance as 0.167, and variance of the autoregressive effect of time as 0.002. The generalized (Poisson) mixed model for each count response (J2 and eggs) was fitted using the "lme4" library from R package. Analysis of eggs count in the roots included fresh root mass as a co-variable.

Analysis of variance (ANOVA) was performed to verify the factors that significantly affected the galls index, lettuce and garlic production per plant, total gross and net income, as well as SOD, CAT, APX, H_2O_2 and lipid peroxidation in lettuce and garlic leaves.

Multivariate analysis (MANOVA) was performed on lettuce and garlic foliar nutrients content to test for differences between the factors: *B. subtilis* BV09 application, garlic cultivation (intercropped with lettuce) or lettuce cultivation (intercropped with garlic), as well as their interaction, using the Pillai's Trace test, with significance level of 5% (Pillai, 1955).

For the analysis of production per lettuce plant, data were transformed into logarithmic form, while for the analysis of CAT in lettuce leaves, garlic production per plant and galls index, data were transformed into square root, which were the most suitable. For the analysis of lipid peroxidation in garlic leaves and manganese (Mn) in lettuce leaves the Box-Cox transformation was applied. All square root and logarithmic transformations passed a normality test using the power series transformation of Box & Cox (1964).

3. Results

3.1 *Meloidogyne incognita* population over time

Variations over time in the number of *M. incognita* second-stage juveniles (J2) in the soil were observed (Fig. 2). While different strategies for the management of *M. incognita* were being performed, from April 15 to September 8 (0 to 146 days after planting), there was a reduction in J2 population in the soil in all treatments. Until the 146 DAP, while lettuce plants were being harvested, their roots were being ripped out from the soil and removed from

the productive area. The removal of infested roots was efficient in reducing the population of J2 in the soil, reducing the population even in the plots where lettuce was cultivated alone (T1: lettuce monoculture) without any other treatment for the management of *M. incognita*.

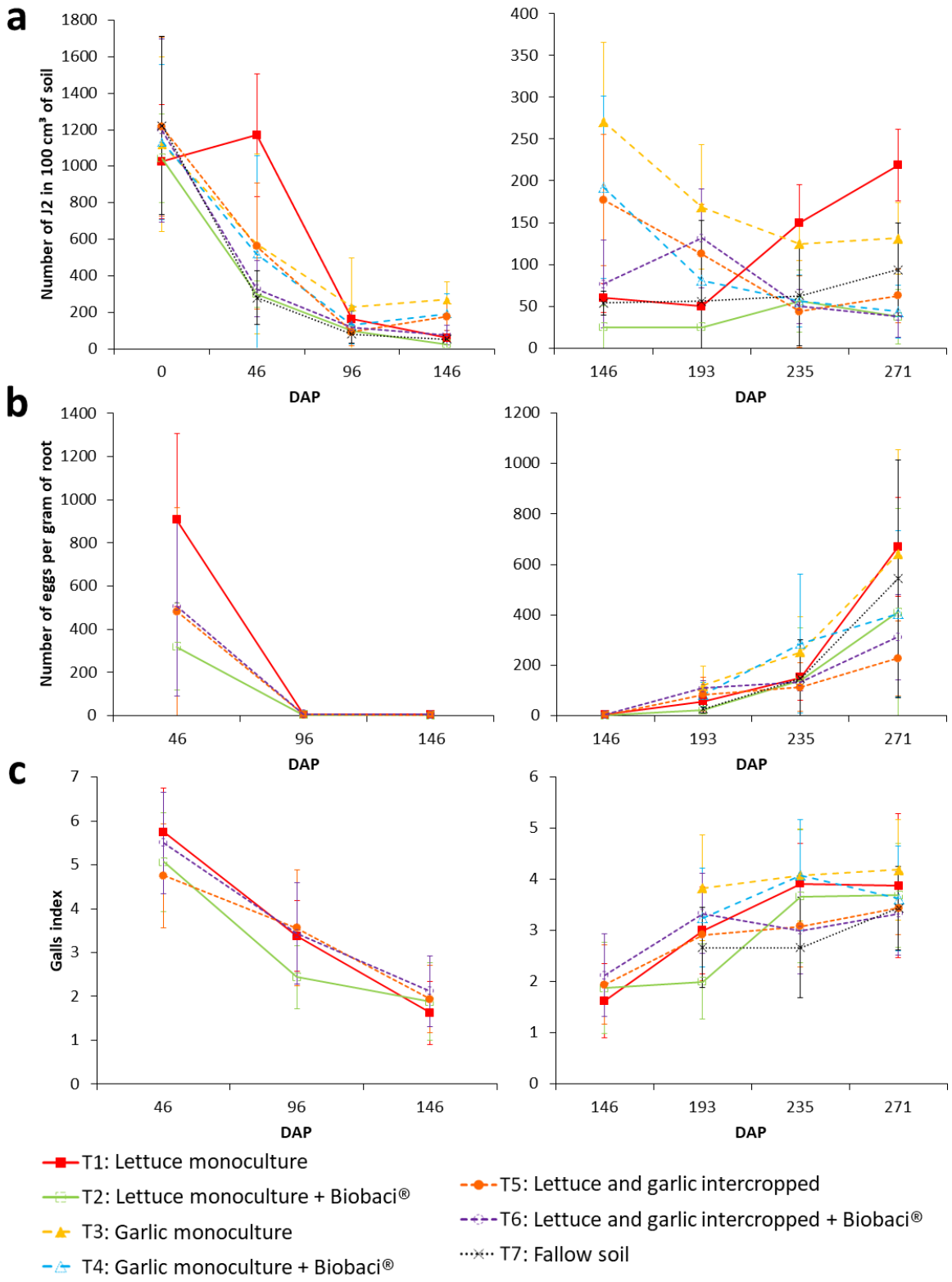

Fig. 2. *Meloidogyne incognita* population over time. **a**) population of second-stage juveniles (J2) in the soil, **b**) *M. incognita* eggs populations and **c**) galls index on lettuce roots over time in the experimental plots subjected to the following treatments: T1: lettuce monoculture; T2:

lettuce monoculture with the application of *B. subtilis* BV09; T3: garlic monoculture; T4: garlic monoculture with the application of *B. subtilis* BV09; T5: lettuce intercropped with garlic; T6: lettuce intercropped with garlic and with the application of *B. subtilis* BV09; T7: fallow soil. At 146 days after planting (DAP), after garlic harvest, lettuce was planted in monoculture in all plots.

Lettuce monoculture (T1) was the only treatment that showed an increase of approximately 14% in J2 number in the soil at 46 days after planting (DAP), when the first harvest of lettuce was performed. This result confirm the lettuce plants susceptibility (reproductive factor (RF) = 8.08 ± 3.69) observed in greenhouse assay with the evaluations performed at 60 days of the introduction of *M. incognita* in pots. After removing lettuce roots in the first harvest, along with the lower temperatures in June and July (Fig. S1), there was a significant reduction (approximately 86%) in J2 number in the soil at 96 DAP, second harvest of lettuce monoculture (T1).

At 46 and 93 DAP, garlic cultivation (T3 and T4) promoted a significant reduction in J2 number in the soil, confirming garlic plants resistance (RF = 0.07 ± 0.02) observed in greenhouse assay, in which the evaluation was performed in garlic plants with 97 days of growth at 60 days of the introduction of *M. incognita* in pots. However, from 93 to 146 DAP there was an increase in J2 number in the soil during garlic cultivation (Fig. 3).

The result of using different strategies for the management of *M. incognita* at 146 DAP and their residual effect up to 271 DAP were evaluated, with only the application of *B. subtilis* BV09 been carried out until 271 DAP in the respective treatments. For the number of second-stage juveniles (J2) in the soil, the treatments and the interaction of treatments with time were significant (Table S1). A simplified model involving only the relevant fixed effects (Table S2) was compared with the complete model by the Chi-square test, which indicated that there was no difference between them and, therefore, it was possible to use the simplest model.

The presence of *Bacillus subtilis* BV09 was the most significant factor in this analysis for the J2 count in the soil, being also significant the interactions of garlic with time and garlic with lettuce (Table S1 and Table S3). The application of *B. subtilis* BV09 led to a reduction in the J2 count in the soil, being observed the smaller marginal mean for the J2 count in the treatment T2, where only lettuce was cultivated with the application of *B. subtilis* BV09 (Fig. S2 and Table 1).

For the interaction of garlic and time, only the linear effect was significant (Table S3). From 146 to 271 DAP, a reduction in the J2 count in the soil was observed in the plots where

garlic was cultivated (Fig. S3 and Table 2), while the J2 count in the soil started to increase in lettuce monoculture (T1) and fallow soil (T7) (Fig. 2).

For the interaction of lettuce and garlic, smaller marginal mean for the J2 count was observed in the intercropping of lettuce with garlic than in garlic monoculture without the application of *B. subtilis* BV09. The intercropping of lettuce with garlic and with the application of *B. subtilis* BV09 (T6) presented a marginal mean for the J2 count in the soil very close to the observed in the fallow soil (T7), without cropping until 146 DAP (Fig. S2 and Table 1).

Table 1. Marginal means of *M. incognita* J2 in 100 cm³ of soil observed in the different treatments, based on the more parsimonious Poisson mixed-effects model.

Lettuce	Garlic	Bsubt[a]	Mean	SE[b]	LCL[c]	UCL[d]
1	0	0	118.3	21.85	82.35	170
1	**0**	**1**	**39.45**	**11.25**	**22.55**	**69**
0	1	0	170.7	27.3	124.75	233.5
0	1	1	95.5	18.85	64.85	140.5
1	1	0	98.4	19.2	67.15	144
1	1	1	78.1	16.7	51.4	119
0	0	0	70.45	15.8	45.4	109.5

[a]*Bacillus subtilis* BV09; [b]standard error; [c]lower confidence limits; [d]upper confidence limits.

Table 2. Marginal means of *M. incognita* J2 in 100 cm³ of soil, observed at 146, 193, 235, and 271 days after planting (DAP) in plots where garlic was planted, based on the more parsimonious Poisson mixed-effects model.

Time (DAP[a])	Garlic	Mean	SE[b]	LCL[c]	UCL[d]
146	1	175	27.8	128.1	239
193	1	130.5	22.95	92.35	184
235	1	74	16.3	48.2	114
271	1	74	16.3	48.2	114

[a]Days after planting; [b]standard error; [c]lower confidence limits; [d]upper confidence limits.

In the first cycle of lettuce planting, high reproduction of *M. incognita* was observed, mainly in the monoculture of lettuce without the application of *B. subtilis* BV09 (T1), with high galls index and high number of eggs per gram of root at 46 DAP in the first lettuce harvest. After ripping out the infested roots from the soil and removing them from the productive area in the first harvest (at 46 DAP), in the second lettuce harvest (at 96 DAP) there was a marked reduction in the galls index and number of eggs per gram of lettuce root. The galls index kept decreasing until 146 DAP, in the third lettuce harvest and first harvest of garlic, while the number of eggs per gram of lettuce root remained low (Fig. 2).

After completion of treatments for the management of *M. incognita* (at 146 DAP) was

399 noted that the galls index and the number of eggs per gram of lettuce roots started to increase
400 in all treatments from 193 DAP (fourth lettuce harvest) (Fig. 2), when the temperature rose up
401 (Fig. S1).

402 For the eggs count in lettuce roots, the time and the interactions of treatments with
403 time were significant (Table S4). The effect of fresh root mass co-variable was significant for
404 eggs count in lettuce roots (Table S4). A simplified model involving only the relevant fixed
405 effects was compared with the complete model by the Chi-square test, which indicated that
406 there was no difference between them and, therefore, it was possible to use the simplest model
407 (Table S5).

408 For the interaction of garlic with time, the linear and cubic effects were significant
409 (Table S6). The eggs count increased over time from 146 to 235 DAP, presenting a reduction
410 at 271 DAP in the plots where garlic was cultivated (Fig. S4**a**). For the interaction of lettuce
411 with time, the linear and quadratic effects were significant. In the plots where the lettuce was
412 cultivated, the eggs count increased over time from 146 to 235 DAP, presenting a subtle
413 reduction at 271 DAP (Fig. S4**b**).

414 In plots where *B. subtilis* BV09 was applied there was a reduction in the egg count
415 from 235 to 271 DAP (Fig. S4**c**). The fallow soil (T7) and the application of *B. subtilis* BV09
416 in lettuce monoculture (T2) showed smaller marginal means for the eggs count in lettuce roots
417 compared to the other treatments (Table 3). The interaction of lettuce with garlic caused a
418 reduction in the eggs count in lettuce roots at 271 DAP (Fig. S4**d**).

419

420 **Table 3.** Marginal means for the number of *M. incognita* eggs per gram of lettuce roots
421 observed in the different treatments, based on the more parsimonious Poisson mixed model.

Lettuce	Garlic	Bsubt[a]	Mean	SE[b]	LCL[c]	UCL[d]
1	0	0	27.14	8.97	14.2	51.9
1	**0**	**1**	**7.96**	**2.76**	**4.04**	**15.7**
0	1	0	34.44	11.36	18.04	65.8
0	1	1	20.47	6.8	10.67	39.3
1	1	0	24.65	8.06	12.98	46.8
1	1	1	30.34	9.86	16.04	57.4
0	**0**	**0**	**5.16**	**1.83**	**2.57**	**10.4**

422 [a]*Bacillus subtilis* BV09; [b]standard error; [c]lower confidence limits; [d]upper confidence limits.
423

424 For the galls index in lettuce roots, the time and the presence of the factors garlic or *B.*
425 *subtilis* BV09 alone, as well the interaction of lettuce and garlic or interaction of garlic and
426 time had a significant effect ($p < 0.05$) (Table S7).

427 For the interaction of garlic and time, the galls index increased quickly from 146 to
428 193 DAP. From 193 to 271 DAP it continued to increase more mildly (Fig. S5**a**). In plots

where lettuce was cultivated, the galls index continuously increased over time from 146 to 271 DAP (Fig. S5**b**). The galls index in lettuce roots presented a reduction as an effect of the application of *B. subtilis* BV09 (Table S8), which was more evident at 193 and 271 DAP (Fig. S5**c** and Table 4). In lettuce monoculture, *B. subtilis* BV09 application (T2) showed galls index significantly smaller than "no application" (T1) at 193 DAP (Table 4).

The treatments with combinations of lettuce and garlic interact with time resulting in higher galls index in lettuce roots than lettuce alone at 146 and 193 DAP, and smaller galls index than garlic alone at 193 DAP. At 235 and 271 DAP the galls index for lettuce intercropped with garlic treatment was smaller than lettuce or garlic alone (Fig. S5**d** and Table 4).

Table 4. Averages of galls index in lettuce roots observed in the different treatments over time (days after planting), based on the linear model for the randomized complete block design.

DAP[a]	Lettuce	Garlic	Bsubt[b]	Average	SE[c]	LCL[d]	UCL[e]
146	1	0	0	1.25	0.074	1.06	1.43
146	1	0	1	1.33	0.074	1.15	1.52
146	0	1	0	*NA*[f]	*NA*	*NA*	*NA*
146	0	1	1	*NA*	*NA*	*NA*	*NA*
146	1	1	0	1.36	0.074	1.18	1.55
146	1	1	1	1.43	0.074	1.24	1.62
146	0	0	0	*NA*	*NA*	*NA*	*NA*
193	1	0	0	1.72	0.077	1.56	1.87
193	**1**	**0**	**1**	**1.39**	**0.077**	**1.23**	**1.55**
193	0	1	0	1.94	0.077	1.78	2.1
193	0	1	1	1.78	0.077	1.63	1.94
193	1	1	0	1.69	0.077	1.53	1.85
193	1	1	1	1.81	0.077	1.66	1.97
193	0	0	0	1.62	0.077	1.46	1.78
235	1	0	0	1.97	0.118	1.52	2.42
235	1	0	1	1.89	0.118	1.44	2.34
235	0	1	0	2.01	0.118	1.56	2.46
235	0	1	1	2	0.118	1.55	2.46
235	1	1	0	1.74	0.118	1.29	2.19
235	1	1	1	1.72	0.118	1.26	2.17
235	0	0	0	1.61	0.118	1.15	2.06
271	1	0	0	1.94	0.065	1.81	2.07
271	1	0	1	1.9	0.065	1.77	2.03
271	0	1	0	2.03	0.065	1.90	2.16
271	0	1	1	1.89	0.065	1.76	2.02
271	1	1	0	1.85	0.065	1.72	1.98
271	1	1	1	1.81	0.067	1.68	1.95
271	0	0	0	1.84	0.065	1.71	1.97

[a]Days after planting; [b]*Bacillus subtilis* BV09; [c]standard error; [d]lower confidence limits; [e]upper confidence limits; [f]not applicable, because there was no lettuce plant.

3.2 Lettuce and garlic production

The production per lettuce plant had a significant effect ($p < 0.05$) of the time, as well as the interaction of time with garlic and time with lettuce and garlic (Table S9). For the time factor, the quadratic, cubic and fourth order effects were significant on lettuce production, while for the interaction of garlic and time, the fourth and fifth order effects were significant (Table S10). The production per garlic plant had a significant effect ($p < 0.05$) of the presence of the factor lettuce, as well as the interaction of lettuce with *B. subtilis* BV09 (Table S11). The application of *B. subtilis* BV09 anole had no significant effect ($p > 0.05$) on the production per plant of lettuce or garlic (Table S9 and Table S11).

For lettuce, the production per plant oscillated over the 271 days but not among the treatments, except at 146 DAP when the production per lettuce plant was smaller in the presence of garlic (lettuce intercropped with garlic) than in its absence (lettuce monoculture) (Fig. 3 and Table S12). Lettuce plants harvested at 146 DAP are presented in Fig. 4.

For garlic, the production per plant was higher in the presence of lettuce (garlic intercropped with lettuce) and *B. subtilis* BV09 than in the absence of these factors (garlic monoculture) (Fig. 3 and Table S13). Garlic bulbs harvested at 146 DAP are presented in Fig. 4.

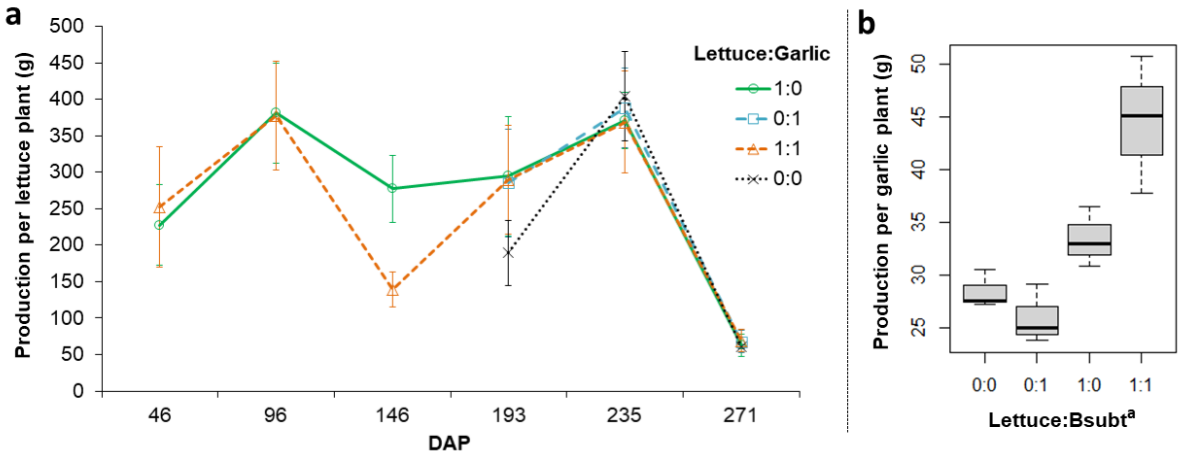

Fig. 3. Effect of lettuce and garlic cultivation on the production per lettuce plant over time (**a**) and the production per garlic plant at 146 days after planting (DAP) (**b**). Lettuce and garlic monoculture, lettuce intercropped with garlic, and fallow soil were performed during the first 146 DAP. Then, from 147 to 271 DAP, lettuce monoculture was carried out in all plots. [a]*B. subtilis* BV09. In graphs subtitles: 0 = presence and 1 = absence of the factors.

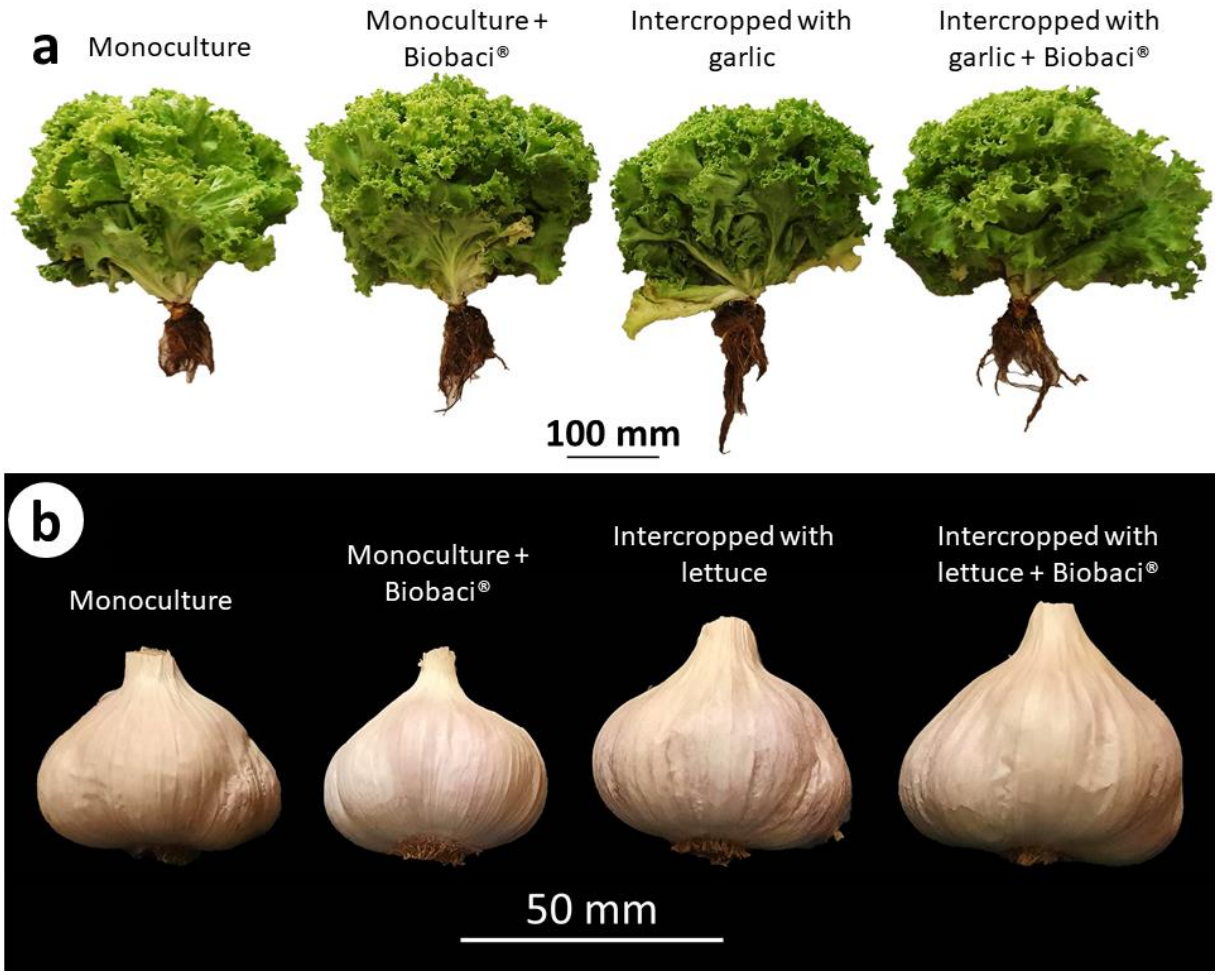

Fig. 4. Lettuce plants (**a**) and garlic bulbs (**b**) harvested at 146 DAP after they have been cultivated under different treatments. T1: lettuce monoculture; T2: lettuce monoculture with the application of *B. subtilis* BV09; T3: garlic monoculture; T4: garlic monoculture with the application of *B. subtilis* BV09; T5: lettuce intercropped with garlic; T6: lettuce intercropped with garlic and with the application of *B. subtilis* BV09.

3.3 Total gross and net income from lettuce and garlic production

The presence of the factor lettuce, as well as the interaction of lettuce with garlic had a significant effect ($p < 0.001$) on the estimated total gross and net income (Table S14). Higher total gross and net incomes were found in the lettuce monoculture (T1 and T2) and intercropping of lettuce with garlic (T5 and T6), independent of the application of *B. subtilis* BV09. In the fallow soil (T7) and in garlic monoculture (T3 and T4) the lowest total gross and net income were shown, which occurred because they were treatments in which there was no lettuce productivity during the first 146 days of the experiment (Fig. 5 and Table S15).

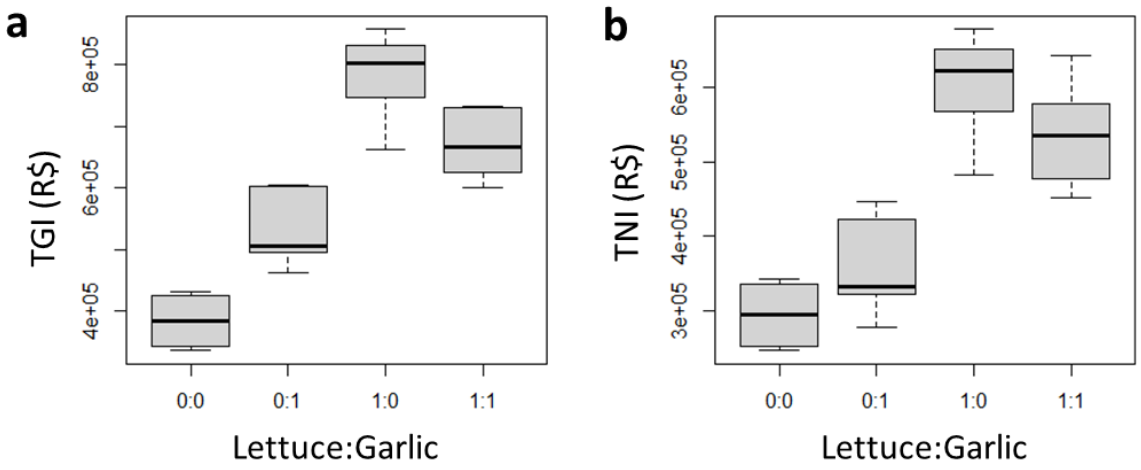

Fig. 5. Effect of lettuce and garlic cultivation on estimated total gross (**a**) and net (**b**) income over the period of 271 days. Values were estimated for one hectare. Lettuce monoculture (1:0), garlic monoculture (0:1), lettuce intercropped with garlic (1:1), and fallow soil (0:0) were performed during the first 146 DAP. Then, from 147 to 271 DAP, lettuce monoculture was carried out in all plots. TGI: total gross income in reais (R$); TNI: total net income in reais (R$). In graphs subtitles: 0 = presence and 1 = absence of the factors.

3.4 Biochemical defense response

In lettuce leaves, superoxide dismutase (SOD) had a significant effect ($p < 0.05$) of the interaction of garlic and time. Ascorbate peroxidase (APX) had a significant effect ($p < 0.05$) of time. Lipid peroxidation had a significant effect ($p < 0.05$) of the factors time and garlic, as well as the double interaction of time and garlic or the triple interaction of time, garlic and *B. subtilis* BV09 application (Table S16). Catalase (CAT) and hydrogen peroxide (H_2O_2) in lettuce leaves had no significant effect ($p > 0.05$) of the treatments. Lettuce leaves showed smaller SOD in the presence of garlic at 144 DAP when compared to the value observed at 43 DAP, and smaller APX at 144 DAP than at 43 DAP. Lipid peroxidation was higher at 144 DAP than at 43 DAP, except in the absence of garlic and *B. subtilis* BV09 which presented no difference over time (Table 5).

Table 5. Marginal means of superoxide dismutase (SOD), ascorbate peroxidase (APX) and lipid peroxidation in lettuce leaves observed in the different treatments over time, based on the linear model for the randomized complete block design.

DAP[a]			APX (μM H_2O_2 min^{-1} mg^{-1} FW[b])			
			Mean	SE[c]	LCL[d]	UCL[e]
43			2.98	0.28	2.39	3.56
144			2.04	0.28	1.46	2.62
DAP	**Garlic**		**SOD** (SOD units mg^{-1} FW)			
			Mean	**SE**	**LCL**	**UCL**
43	0		0.43	0.03	0.37	0.49
144	0		0.45	0.03	0.39	0.51
43	1		0.47	0.03	0.41	0.53
144	1		0.35	0.03	0.29	0.41
DAP	**Garlic**	**Bsubt[f]**	**Lipid peroxidation** (nmol MDA mg^{-1} FW)			
			Mean	**SE**	**LCL**	**UCL**
43	0	0	196	7.66	180	212
144	0	0	192	7.66	176	208
43	1	0	167	7.66	151	183
144	1	0	271	7.66	255	287
43	0	1	168	7.66	152	184
144	0	1	232	7.66	216	248
43	1	1	187	7.66	171	203
144	1	1	250	7.66	235	266

[a]Days after planting; [b]fresh weight; [c]standard error; [d]lower confidence limits; [e]upper confidence limits; [f]*Bacillus subtilis* BV09.

In garlic leaves, H_2O_2 had a significant effect ($p < 0.05$) of the time and lipid peroxidation had a significant effect ($p < 0.05$) of the factors time and lettuce, as well as the double interactions of time and *B. subtilis* BV09 application or double interaction of lettuce and *B. subtilis* BV09 application (Table S17). SOD, APX and CAT in garlic leaves had no significant effect ($p > 0.05$) of the treatments. Garlic leaves showed higher H_2O_2 at 144 DAP than at 43 DAP. Lipid peroxidation was higher at 144 DAP than at 43 DAP, except in the presence of lettuce and *B. subtilis* BV09 which presented no difference over time (Table 6).

Table 6. Marginal means of hydrogen peroxide (H_2O_2) and lipid peroxidation in garlic leaves observed in the different treatments over time, based on the linear model for the randomized complete block design.

DAP[a]			H_2O_2 (µmol H_2O_2 mg^{-1} FW[b])			
			Mean	SE[c]	LCL[d]	UCL[e]
43			0.736	0.0572	0.617	0.854
144			1.248	0.0572	1.129	1.366
DAP	**Lettuce**	**Bsubt[f]**	**Lipid peroxidation** (nmol MDA mg^{-1} FW)			
			Mean	SE	LCL	UCL
43	0	0	249	15.9	216	282
144	0	0	360	15.9	327	393
43	1	0	276	15.9	243	308
144	1	0	368	15.9	336	401
43	0	1	246	15.9	214	279
144	0	1	274	15.9	241	307
43	1	1	342	15.9	310	375
144	1	1	338	15.9	305	371

[a]Days after planting; [b]fresh weight; [c]standard error; [d]lower confidence limits; [e]upper confidence limits; [f]*Bacillus subtilis* BV09.

3.5 Foliar nutrient content

Lettuce and garlic foliar nutrient content were evaluated at 146 DAP, at the end of carrying out the *M. incognita* management strategies to verify if there was an effect of treatments on plant nutrition. MANOVA statistics (Pillai's trace, F, and p values) showed that garlic foliar nutrients content did not differed significantly (Table S18), while when evaluating each nutrient separately the lettuce cultivation had a significant ($p < 0.05$) effect on nitrogen (N), phosphorus (P) and potassium (K) content in garlic leaves (Table S19).

MANOVA statistics (Pillai's trace, F, and p values) showed that lettuce foliar nutrients content differed significantly due to garlic cultivation (intercropped with lettuce), as well as due to the interaction of garlic cultivation (intercropped with lettuce) and *B. subtilis* BV09 application (Table S20). When evaluating each nutrient separately, garlic cultivation had a significant ($p < 0.05$) effect on P content in lettuce leaves (Table S21).

Garlic cultivation caused a significant ($p < 0.05$) reduction on P content in lettuce leaves in relation to control (Table 8), while lettuce cultivation caused a significant ($p < 0.05$) increase on N, P and K in garlic leaves in relation to control (Table 7). For the other macro- and micronutrients in lettuce or garlic leaves, there was no significant effect of garlic or lettuce cultivation, nor the application of *B. subtilis* BV09 or their interaction.

Table 7. Garlic foliar nutrients content estimates. The intercept is garlic leaves in monoculture, with no lettuce cultivation nor *B. subtilis* BV09 application.

	N[b]	**P**[c]	**K**[d]	**Ca**[e]	**Mg**[f]	**S**[g]	**B**[h]	**Cu**[i]	**Fe**[j]	**Mn**[k]	**Zn**[l]
Intercept	13.25	2.00	26.08	13.85	2.68	3.88	33.47	5.98	260.90	30.20	14.32
Lettuce	**2.35**	**0.23**	**2.65**	1.10	-0.13	0.25	-5.25	-0.33	-33.25	-4.63	5.48
Bsubt[a]	-0.10	0.10	-0.58	-0.93	-0.33	0.10	-3.35	-0.40	-68.38	-1.93	2.48
Lettuce:Bsubt	0.73	-0.10	0.50	-0.10	0.18	0.00	3.38	0.50	88.80	3.00	-4.55

[a]*Bacillus subtilis* BV09; [b]nitrogen (g/Kg); [c]phosphorus (g/Kg); [d]potassium (g/Kg); [e]calcium (g/Kg); [f]magnesium (g/Kg); [g]súlfur (g/Kg); [h]boron (mg/Kg); [i]copper (mg/Kg); [j]iron (mg/Kg); [k]manganese (mg/Kg); [l]inc (mg/Kg).

Table 8. Lettuce foliar nutrients content estimates. The intercept is lettuce leaves in monoculture, with no garlic cultivation nor *B. subtilis* BV09 application.

	N[b]	**P**[c]	**K**[d]	**Ca**[e]	**Mg**[f]	**S**[g]	**B**[h]	**Cu**[i]	**Fe**[j]	**Mn**[k]	**Zn**[l]
Intercept	24.48	4.93	45.32	9.80	3.08	2.98	29.30	9.70	1074.00	132.80	46.20
Garlic	-2.93	**-0.58**	-3.15	1.00	0.13	-0.08	-1.23	-1.05	-68.52	17.97	3.50
Bsubt[a]	-0.88	0.33	-0.65	-0.40	0.05	0.00	1.35	0.45	31.35	-11.38	7.03
Garlic:Bsubt	0.55	-0.25	4.70	0.78	-0.08	0.03	-2.15	0.23	342.40	22.40	-13.22

[a]*Bacillus subtilis* BV09; [b]nitrogen (g/Kg); [c]phosphorus (g/Kg); [d]potassium (g/Kg); [e]calcium (g/Kg); [f]magnesium (g/Kg); [g]súlfur (g/Kg); [h]boron (mg/Kg); [i]copper (mg/Kg); [j]iron (mg/Kg); [k]manganese (mg/Kg); [l]inc (mg/Kg).

4. Discussion

The results obtained highlight the importance of carrying out integrated management to control *M. incognita* in lettuce crops in the field, combining garlic cultivation, application of *B. subtilis* BV09 (microbiological nematicide) and removal of infested roots from the production area to achieve a balance among *M. incognita* population, productivity and net income of lettuce and garlic crops.

The practice of removing and destroying infected crop residues (sanitization) is efficient in reducing RKN population in the place, as it eliminates the nematodes and eggs that are inside the roots preventing its multiplication after harvest, since nematodes of the genus *Meloidogyne* are obligate parasites of plants (Bridge, 1996; Collange et al., 2011). In this work, the efficiency of removing and destroying infected roots was quantified, reducing by approximately 86% of RNK population in the soil when performed for the first time, and being as efficient as fallow or the application of the microbiological nematicide during its execution until 146 DAP (Fig. 2).

Garlic cultivation showed a residual effect keeping *M. incognita* population at a lower level during three lettuce crop cycles, until 4 months after garlic harvest, while during the same time *M. incognita* population started to increase in the control plots (lettuce monoculture without *B. subtilis* BV09 application) (Fig. 2). Garlic residual effect reducing the *M. incognita* population when cultivating a susceptible crop in succession in the same rows where garlic

had been previously cultivated (El-Saedy et al., 2014), as well as the intercropping systems with garlic (*Allium sativum*) (Seman et al., 2020) or other *Allium* species (Abdel-Baset and Allah, 2020; Detrey et al., 2022; Nie et al., 2021) used for the management of *Meloidogyne* spp. are found in the literature. Garlic cultivation proved to be efficient for the management of *M. incognita* on lettuce crop in the field both through its monoculture and in intercropping systems. Garlic is a plant with nematicidal properties (Eder et al., 2021; Jardim et al., 2020; Kouamé, 2021), which grows in cold weather (Choudhary et al., 2022), being unfavorable for *M. incognita* reproduction since this nematode life cycle is longer under these conditions (Ploeg and Maris, 1999), and showed a resistant reaction (RF < 1) to *M. incognita* in the greenhouse assay. However, garlic crop cycle lasting about five months (± 150 days) had been enough time to start occurring the reproduction of *M. incognita* in its root system, being observed from 93 to 146 DAP an increase in the number of J2 in the soil (Fig. 2). Therefore, garlic cultivation in *M. incognita* infested area should be carried out together with other strategies in an integrated management. Intercropping lettuce with garlic plants, with and without the application of *B. subtilis* BV09, exhibited better results in reducing *M. incognita* population compared to garlic monoculture without applications (Table 1 and Fig. S2). This result may be related to the removal of infested roots in the lettuce rows during garlic growth, reducing *M. incognita* inoculum compared to garlic monoculture, where *M. incognita* reproduction was occurring even at a low reproductive factor.

The application of *Bacillus subtilis* BV09 was an efficient strategy to reduce *M. incognita* population in the field and helped keep it at a lower level in critical periods when *M. incognita* population increased in lettuce monoculture with no applications (Fig. 2 and Table 3). Several studies have been confirming the effectiveness of *B. subtilis* in controlling *M. incognita* (Adam et al., 2014; Basyony and Abo-Zaid, 2018; Cao et al., 2019; Hoffmann-Hergarten et al., 1998; Hussain et al., 2020). This bacteria can act as bionematicide (Hussain et al., 2020), repelling or killing second-stage juveniles (J2) and inhibiting egg hatching (Cao et al., 2019; Dawar et al., 2008), as well as acting indirectly in the control of plant-parasitic nematode by promoting resistance induction in plants (Adam et al., 2014).

The practice of keeping the soil under fallow and irrigated reduced *M. incognita* population in the field (Table 1 and Fig. S2). This occurred because irrigation induces the hatching of second-stage juveniles (J2) that are already formed in the eggs and the absence of hosts increases J2 mortality in the soil since they are obligate parasites and found no place to stay alive and reproduce (Dutra and Campos, 2003; Evlice et al., 2022). However, when cultivating lettuce, which is a susceptible species, the population of *M. incognita* gradually

increased again. Therefore, there was a residual effect due to the low initial inoculum, but this residual effect is not very long-lasting (Evlice et al., 2022).

The lettuce monoculture and intercropping of lettuce with garlic showed greater gross and net income, over the 271 days of the experiment, than garlic monoculture and fallow (Fig. 5). Diversifying crops through intercropping lettuce with garlic was important to manage *M. incognita* along with achieving high income. Crop diversification through intercropping may provide many benefits, such as increase in biological diversity causing suppressive effects on diseases, pests and weeds, in addition to less chance of complete failure of crop, better use of resources and increase in net returns, mainly when growing two cash crops, improving the acceptance of producers in carrying out the intercropping (Hasanuzzaman, 2019). More studies are needed to assess the effect of garlic planting density when intercropped with lettuce to achieve RKN control and increase the productivity and income of lettuce and garlic crops (Hata et al., 2019; Lai et al., 2020). Another advantage of performing the intercropping of lettuce with garlic in autumn and winter is that lettuce consumption is smaller during the coldest seasons. Thus, reducing the number of lettuce plants to cultivate garlic in the same agricultural area may reduce loss and increase producers' income, in addition to helping control the RKN.

In the plots under fallow, the area was kept unproductive while the *M. incognita* population management was performed during most of the experiment and, after that, only three lettuce cropping cycles were carried out reducing significantly the gross and net income. Thus, fallow may be done during shorter periods, such as proposed by Dutra and Campos (2003) to plow and irrigate the soil 14 days before seeding, showing reduction in *M. incognita* population and increasing beans productivity.

The production per lettuce plant oscillated over the 271 days of the experiment but not among the treatments at each harvest time, except at 146 DAP when the production per lettuce plant was smaller in the intercropping of lettuce with garlic than in lettuce monoculture, which may be related to competition for nutrients and solar radiation between lettuce and garlic plants. At 146 DAP garlic plants were large, shading the lettuce plants and, consequently, affecting their growth. The oscillations in the production per lettuce plant over time may be related to several factors, such as *M. incognita* population and weather conditions. Lettuce growth was affected in the first cropping cycle, showing smaller production per plant at 46 DAP independently of the treatment, which may have occurred due to the high *M. incognita* population (± 1,100 J2 in 100 cm^3 of soil) in all plots. The removal of the infested roots at 46 DAP reduced the *M. incognita* population and favored lettuce growth

in the second cropping cycle, increasing the production per plant at 96 DAP. From 235 to 271 DAP, the weather conditions were unfavorable to lettuce growth, presenting low solar radiation and high precipitation, relative humidity, and temperature (Wurr and Fellows, 1991), obtaining the lowest production per plant at 271 DAP in all plots, independently of the treatment (Fig. 3 and Fig. S1).

Although the application of *B. subtilis* BV09 in lettuce monoculture reduced *M. incognita* population in the field compared to lettuce monoculture with no applications (Table 1 and Table 3), it did not cause differences in the production per lettuce plant. This may have happened due to a possible tolerance of lettuce plants of the cultivar Solaris to *M. incognita*, being not observed nematode damage to lettuce shoot growth (Fig. 4), as noted by Cavalcanti et al. (2023).

Garlic intercropping with lettuce and *B. subtilis* BV09 application favored bulb development, showing larger garlic bulbs (Fig. 3 and Fig. 4). In the intercropping system, garlic may have recruited beneficial microorganisms which promoted plant growth and improved bulb development, which was intensified with the application of *B. subtilis* BV09. Beneficial microorganisms, such as bacteria and fungi antagonists to plant pathogens and plant growth promoters, are recruited by the root exudates released into the soil according to plant needs, improving rhizosphere microbiome composition and, consequently, improving plants' health (Sun et al., 2021; Vives-Peris et al., 2020). Increase biodiversity through intercropping systems and the application of biological control agents, such as *B. subtilis* BV09, are some of the agroecological practices that can favor the establishment of beneficial microorganisms in the field, promoting plant growth and increasing soil suppressiveness to RKN (Batista and Singh, 2021; Maitra et al., 2021; Silva et al., 2022).

Under abiotic and biotic stresses, one of the first responses of plant cells is the oxidative burst, in which are generated large amount of reactive oxygen species (ROS) causing oxidative deterioration that may lead to cell death (Bhattacharjee, 2005). To protect themselves from this overproduction of ROS during stress conditions, such as caused by plant-parasitic nematode infection, plants may show enzymatic and non-enzymatic antioxidant defense systems to avoid damage caused by the ROS (Afifi et al., 2014). Host plants infected by *M. incognita* usually show an increase in H_2O_2 levels and lipid peroxidation (Afifi et al., 2014) and, consequently, the superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) enzymes activity can increase during the *M. incognita* infection (Danish et al., 2021). In lettuce leaves, higher SOD and APX activities were observed when the *M. incognita* population was higher (at 43 DAP), reducing the activity of APX when

694 completing the management period of *M. incognita* (at 144 DAP) with a smaller *M. incognita*
695 population in all treatments (Fig. 2), while SOD activity only reduced at 144 DAP in the
696 treatments where the lettuce was intercropped with garlic (Table 5). The lower lipid
697 peroxidation in lettuce leaves when SOD and APX enzymes showed higher activity, during
698 the period when *M. incognita* population was higher, suggests that these enzymes were
699 reducing the potential cellular damage of ROS and, consequently, avoiding lipid peroxidation
700 (Karanastasi et al., 2018). The use of biological control agents against the RKN, such as *B.*
701 *subtilis*, can increase plants' enzymatic and non-enzymatic antioxidant activity, in addition to
702 reducing H_2O_2 and lipid peroxidation (Abbasi et al., 2014; Khanna et al., 2019). At 43 DAP,
703 smaller lipid peroxidation was observed in lettuce plants with the application of *B. subtilis*
704 BV09 or intercropped with garlic when compared to lettuce plants in monoculture with no
705 applications. On the other hand, at 144 DAP, when SOD and APX showed reduced activity,
706 the lipid peroxidation in lettuce leaves was affected by garlic intercropping and *B. subtilis*
707 BV09 application, showing increase even with smaller *M. incognita* population. This result
708 suggests that the lipid peroxidation increase at 144 DAP could be related to another stress that
709 lettuce plants were undergoing, such as competition with garlic plants for solar radiation and
710 nutrients, since at the end of their cycle the garlic plants were large and shading the lettuce
711 plants.

712 In garlic leaves, there was an increase in H_2O_2 levels and lipid peroxidation at the end
713 of its cropping cycle (at 144 DAP), which may be related to a stress in garlic plants due to the
714 increase in *M. incognita* population in their roots over time. Furthermore, there was no
715 significant increase in lipid peroxidation from 43 to 144 DAP in garlic leaves when *B. subtilis*
716 BV09 was applied, thus, its application may help protecting garlic plant from the stress
717 caused by *M. incognita* infection (Table 6).

718 There was not much difference between the treatments in nutritional composition of
719 lettuce and garlic leaves when completing the period of *M. incognita* management (at 146
720 DAP). This may be related to the small *M. incognita* population in all treatments in this
721 period, not showing symptoms of nematode infection since there was no decreasing in the
722 overall nutrient content (Melakeberhan et al., 1987). On the other hand, there was probably
723 competition between lettuce and garlic for some macronutrients since in intercropping
724 treatment lettuce leaves showed a reduction in P content (Table 8), while garlic leaves showed
725 an increase in N, P and K content (Table 7).

726

727 **5. Conclusions**

Garlic cultivation and the application of *Bacillus subtilis* BV09 were efficient in the integrated management of *Meloidogyne incognita* in lettuce crops, reducing the nematode population in the field. The production per garlic plant was improved by intercropping with lettuce and *B. subtilis* BV09 application, while the production per lettuce plant on its third cropping cycle was affected by the intercropping when garlic plants were large. Competition between lettuce and garlic during the final stage of the intercropping affected their foliar nutrient content. Intercropping enabled achieving high total income from lettuce and garlic crops, as the total income obtained only from lettuce monoculture. The *M. incognita* attack activated plants' biochemical defense response, while the application of *B. subtilis* BV09 and the intercropping system helped to protect lettuce and garlic plants against the stress caused by the *M. incognita* infection. The removal of infested roots presented considerable results in the management of RKN in lettuce crops. Fallow soil was also efficient in reducing *M. incognita* population, however, total income was damaged when leaving the area unproductive for a long period.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. The authors extend thanks to the Federal University of Lavras (UFLA) and Technology Development and Transfer Center (CDTT) for the structure provided to carry out the experiments and for the support of the technicians and professors. Thanks to the institutions: FAPEMIG, CNPq, and CAPES for providing the necessary equipment and technical support for the experiments. Thanks to Vittia for providing the Biobaci® and technical support. Thanks to 3rlab for granting discount on foliar analyses. Thanks to Clerio Rodrigues Ribeiro, Sansão Augusto Germano, Otávio Bernardes Machado, Natalia Bernardes Machado, Regiane Alves Belizario, Kamilly Maria Fernandes Fonseca, Brenda Miriam Silva, Maria Luiza Paiva de Oliveira, Lílian Ferreira de Sousa, Ana Luiza Pereira Ferreira and Arthur Metzker for assisting in field measurements.

References

Abbasi, M.W., Ahmed, N., Zaki, M.J., Shuakat, S.S., Khan, D., 2014. Potential of Bacillus species against Meloidogyne javanica parasitizing eggplant (Solanum melongena L.) and induced biochemical changes. Plant Soil 375, 159–173. https://doi.org/10.1007/s11104-013-1931-6

Abd-Elgawad, M.M.M., 2020. Plant-Parasitic Nematodes and Their Biocontrol Agents: Current Status and Future Vistas, in: Ansari, R.A., Rizvi, R., Mahmood, I. (Eds.), Management and Future Recent Advances of Phytonematodes: Challenges. Springer, pp.

171–203.
Abdel-Baset, S.H., Allah, A.M.A., 2020. Effect of Intercropping Onion with Sugar Beet on Productivity of Both Crops and Root-Knot Nematodes Control under Different Onion Plant Densities and Slow-Release N Fertilizer Rates. J. Plant Prod. Sci. 9, 61–75. https://doi.org/10.21608/jpps.2020.157286
Adam, M., Heuer, H., Hallmann, J., 2014. Bacterial Antagonists of Fungal Pathogens Also Control Root-Knot Nematodes by Induced Systemic Resistance of Tomato Plants. PLoS One 9, e90402. https://doi.org/10.1371/journal.pone.0090402
Afifi, A.M.R., Al-Sayed, A.A., Mahfoud, N.A.M., Farahat, A.A., 2014. Enzymatic and non-enzymatic oxidants and antioxidants involved in defense mechanisms against root-knot, reniform and citrus nematodes in their hosts. Egypt. J. Agronematology 13, 172–188.
Akhtar, M., 1997. Current options in integrated management of plant-parasitic nematodes. Integr. Pest Manag. Rev. 2, 187–197.
Basyony, A.G., Abo-Zaid, G.A., 2018. Biocontrol of the root-knot nematode, Meloidogyne incognita, using an eco-friendly formulation from Bacillus subtilis, lab. and greenhouse studies. Egypt. J. Biol. Pest Control 28, 87. https://doi.org/10.1186/s41938-018-0094-4
Batista, B.D., Singh, B.K., 2021. Realities and hopes in the application of microbial tools in agriculture. Microb. Biotechnol. 14, 1258–1268. https://doi.org/10.1111/1751-7915.13866
Bhattacharjee, S., 2005. Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transducation in plants. Curr. Sci. 89, 1113–1121.
Biemelt, S., Keetman, U., Albrecht, G., 1998. Re-Aeration following Hypoxia or Anoxia Leads to Activation of the Antioxidative Defense System in Roots of Wheat Seedlings1. Plant Physiol. 116, 651–658. https://doi.org/10.1104/pp.116.2.651
Box, G.E.P., Cox, D.R., 1964. An Analysis of Transformations. J. R. Stat. Soc. Ser. B 26, 211–243. https://doi.org/10.1111/j.2517-6161.1964.tb00553.x
Bridge, J., 1996. NEMATODE MANAGEMENT IN SUSTAINABLE AND SUBSISTENCE AGRICULTURE. Annu. Rev. Phytopathol. 34, 201–225. https://doi.org/10.1146/annurev.phyto.34.1.201
Bridge, J., Page, S.L.J., 1980. Estimation of Root-knot Nematode Infestation Levels on Roots Using a Rating Chart. Trop. Pest Manag. 26, 296–298. https://doi.org/10.1080/09670878009414416
Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation, in: Methods in Enzymology. Elsevier Inc., pp. 302–310. https://doi.org/10.1016/S0076-6879(78)52032-6
Cao, H., Jiao, Y., Yin, N., Li, Y., Ling, J., Mao, Z., Yang, Y., Xie, B., 2019. Analysis of the activity and biological control efficacy of the Bacillus subtilis strain Bs-1 against Meloidogyne incognita. Crop Prot. 122, 125–135. https://doi.org/10.1016/j.cropro.2019.04.021
Cavalcanti, V.P., Santos, A.F. dos, Rodrigues, F.A., Terra, W.C., Araújo, R.C., Ribeiro, C.R., Campos, V.P., Rigobelo, E.C., Medeiros, F.H.V., Dóri, J., 2023. Use of RGB images from unmanned aerial vehicle to estimate lettuce growth in root-knot nematode infested soil. Smart Agric. Technol. 3, 1–9. https://doi.org/10.1016/j.atech.2022.100100
Charchar, J.M., Moita, A.W., 2005. Metodologia para seleção de hortaliças com resistência a nematóides: Alface/Meloidogyne spp. Embrapa, Brasília.
Choudhary, R., Choudhary, A., Chopra, M.L., Mahala, K., Choudhary, S., 2022. Advances production technology of garlic, in: Yadav, G.K., Dadhich, S.K., Bhateshwar, M.C. (Eds.), Recent Innovative Approaches in Agricultural Science. Bhumi Publishing, pp. 175–181.
Collange, B., Navarrete, M., Peyre, G., Mateille, T., Tchamitchian, M., 2011. Root-knot nematode (Meloidogyne) management in vegetable crop production: The challenge of an

agronomic system analysis. Crop Prot. 30, 1251–1262. https://doi.org/10.1016/j.cropro.2011.04.016

Danish, M., Islam, M., Marraiki, N., Shahid, M., Nouf, S., 2021. Physiological and Molecular Plant Pathology Root-knot nematode Meloidogyne incognita induced changes in morpho-anatomy and antioxidant enzymes activities in Trachyspermum ammi (L .) plant : A microscopic observation. Physiol. Mol. Plant Pathol. 116, 101725. https://doi.org/10.1016/j.pmpp.2021.101725

Dawar, S., Tariq, M., Zaki, M.J., 2008. Application of Bacillus species in control of Meloidogyne javanica (treub) chitwood on cowpea and mash bean. Pakistan J. Bot. 40, 439–444.

Detrey, J., Cognard, V., Djian-Caporalino, C., Marteu, N., Doidy, J., Pourtau, N., Vriet, C., Maurousset, L., Bouchon, D., Clause, J., 2022. Growth and root-knot nematode infection of tomato are influenced by mycorrhizal fungi and earthworms in an intercropping cultivation system with leeks. Appl. Soil Ecol. 169, 104181. https://doi.org/10.1016/j.apsoil.2021.104181

Dutra, M.R., Campos, V.P., 2003. Manejo do Solo e da Irrigação como Nova Tática de Controle de Meloidogyne incognita em Feijoeiro. Fitopatol. Bras. 28, 608–614.

Eder, R., Consoli, E., Krauss, J., Dahlin, P., 2021. Polysulfides Applied as Formulated Garlic Extract to Protect Tomato Plants against the Root-Knot Nematode Meloidogyne incognita. Plants 10, 394. https://doi.org/10.3390/plants10020394

El-Saedy, M.A.M., Mokbel, A.A., Hammad, S.E., 2014. Efficacy of plant oils and garlic cultivation on controlling Meloidogyne incognita infected tomato plants. Pakistan J. Nematol. 32, 39–50.

Evlice, E., Toktay, H., Yatkın, G., Erdoğuş, F.D., İmren, M., 2022. Population fluctuations of root - knot nematodes Meloidogyne chitwoodi and M . hapla under field conditions. Phytoparasitica 50, 233–242. https://doi.org/10.1007/s12600-021-00939-3

Giannopolitis, C.N., Ries, S.K., 1977. Superoxide Dismutases: I. Occurrence in Higher Plants. Plant Physiol. 59, 309–314. https://doi.org/10.1104/pp.59.2.309

Hasanuzzaman, M. (Ed.), 2019. Agronomic Crops: Volume 2: Management Practices. Springer, Singapore. https://doi.org/10.1007/978-981-32-9783-8

Hata, F.T., Ursi, M., Maria, V., Paula, T. De, Souza, N.V. De, 2019. Intercropping garlic in strawberry fields improves land equivalent ratio and gross income. Ciência Rural 49, 1–8.

Havir, E.A., McHale, N.A., 1987. Biochemical and Developmental Characterization of Multiple Forms of Catalase in Tobacco Leaves. Plant Physiol. 84, 450–455. https://doi.org/10.1104/pp.84.2.450

Hoffmann-Hergarten, S., Gulati, M.K., Sikora, R.A., 1998. Yield response and biological control of Meloidogyne incognita on lettuce and tomato with rhizobacteria. J. Plant Dis. Prot. 105, 349–358.

Hussain, T., Haris, M., Shakeel, A., Ahmad, G., Ahmad Khan, A., Khan, M.A., 2020. Bio-nematicidal activities by culture filtrate of Bacillus subtilis HussainT-AMU: new promising biosurfactant bioagent for the management of Root Galling caused by Meloidogyne incognita. Vegetos 33, 229–238. https://doi.org/10.1007/s42535-020-00099-5

Hussey, R.S., Barker, K.R., 1973. A comparison of methods for colecting inocula of meloidogyne spp including a new technique. Plant Dis. Report. 57, 1025–1028.

Jardim, I.N., Oliveira, D.F., Campos, V.P., Silva, G.H., Souza, P.E., 2020. Garlic essential oil reduces the population of Meloidogyne incognita in tomato plants. Eur. J. Plant Pathol. 157, 197–209. https://doi.org/10.1007/s10658-020-02000-1

Jenkins, W.R., 1964. A rapid centrifugal-flotation technique for separating nematodes from

soil. Plant Dis. Report. 48, 692.
Karanastasi, E., Kostara, T., Malamos, N., Zervoudakis, G., 2018. Catalase activity, lipid peroxidation, and protein concentration in leaves of tomato infected with Meloidogyne javanica. Nematropica 48, 15–20.
Khanna, K., Jamwal, V.L., Kohli, S.K., Gandhi, S.G., Ohri, P., Bhardwaj, R., Wijaya, L., Alyemeni, M.N., Ahmad, P., 2019. Role of plant growth promoting Bacteria (PGPRs) as biocontrol agents of Meloidogyne incognita through improved plant defense of Lycopersicon esculentum. Plant Soil 436, 325–345.
Kouamé, A.P., 2021. Effectiveness of Garlic and Onion Aqueous Extracts on Tomato Root-Knot Nematodes (Meloidogyne sp.) in the Autonomous District of Yamoussoukro in Central Côte d'Ivoire. Indian J. Pure Appl. Biosci. 9, 24–35. https://doi.org/10.18782/2582-2845.8532
Lai, R., Zhu, C., Bai, Jingjing, Wu, X., Gu, G., Bai, Jianbao, Zhou, T., Wang, D., Hu, H., Lin, T., 2020. Intercropping garlic at different planting times and densities for insect pest or crop yield and value management in tobacco fields. Entomol. Res. 50, 146–154. https://doi.org/10.1111/1748-5967.12421
Macêdo, F.S., Souza, R.J. de, Pereira, G.M., 2006. Controle de superbrotamento e produtividade de alho vernalizado sob estresse hídrico. Pesqui. Agropecuária Bras. 41, 629–635. https://doi.org/10.1590/S0100-204X2006000400012
Machado, A.C.Z., 2022. Bionematicides in Brazil: an emerging and challenging market. Revisão Anu. Patol. Plantas 35–49. https://doi.org/10.31976/0104-038321v280002
Maitra, S., Hossain, A., Brestic, M., Skalicky, M., Ondrisik, P., Gitari, H., Brahmachari, K., Shankar, T., Bhadra, P., Palai, J.B., Jena, J., Bhattacharya, U., Duvvada, S.K., Lalichetti, S., Sairam, M., 2021. Intercropping—A Low Input Agricultural Strategy for Food and Environmental Security. Agronomy 11, 343. https://doi.org/10.3390/agronomy11020343
Malavolta, E., Vitti, G.C., Oliveira, S.A. de, 1997. Avaliação do estado nutricional das plantas: princípios e aplicações, 2nd ed. Potafos, Piracicaba.
Melakeberhan, H., Webster, J.M., Brooke, R.C., D'Auria, J.M., Cackette, M., 1987. Effect of Meloidogyne incognita on Plant Nutrient Concentration and Its Influence on the Physiology of Beans 1. J. Nematol. 19, 324–330.
Nakano, Y., Asada, K., 1981. Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. Plant Cell Physiol. 22, 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232
Nie, C.R., Feng, Y., Cheng, X.H., Cai, Z.Q., 2021. Intercropping with Chinese leek decreased Meloidogyne javanica population and shifted microbial community structure in Sacha Inchi plantation. J. Agric. Sci. 159, 404–413. https://doi.org/10.1017/S0021859621000708
Pillai, K.C.S., 1955. Some New Test Criteria in Multivariate Analysis. Ann. Math. Stat. 26, 117–121.
Ploeg, A.T., Maris, P.C., 1999. Effects of temperature on the duration of the life cycle of a Meloidogyne incognita population. Nematology 1, 389–393.
Ralmi, N.H.A.A., Khandaker, M.M., Mat, N., 2016. Occurrence and control of root knot nematode in crops: A review. Aust. J. Crop Sci. 10, 1649–1654. https://doi.org/10.21475/ajcs.2016.10.12.p7444
Seman, A., Awol, S., Mashilla, D., 2020. Integrated management of Meloidogyne incognita in tomato (Solanum lycopersicum) through botanical and intercropping. African J. Agric. Res. 15, 492–501. https://doi.org/10.5897/AJAR2019.14040
Sgorlon, L.F.F., Silva, E.H.C., Soares, R.S., Borges, H.O., Diniz, G.M.M., Braz, L.T., Soares, P.L.M., 2018. Host status of crispy-leaf lettuce cultivars to root-knot nematodes. Biosci. J. 34, 1319–1325. https://doi.org/10.14393/BJ-v34n5a2018-39387

914 Sikora, R.A., Fernandez, E., 2005. Nematode parasites of vegetables, in: Luc, M., Sikora,
915 R.A., Bridge, J. (Eds.), Plant-Parasitic Nematodes in Subtropical and Tropical
916 Agriculture. CAB International, Wallingford, UK, pp. 319–391.
917 Silva, J.C.P., Nunes, T.C.S., Guimarães, R.A., Pylro, V.S., Costa, L.S.A.S., Zaia, R., Campos,
918 V.P., Medeiros, F.H.V., 2022. Organic practices intensify the microbiome assembly and
919 suppress root-knot nematodes. J. Pest Sci. (2004). 95, 709–721.
920 https://doi.org/10.1007/s10340-021-01417-9
921 Singh, S., Singh, B., Singh, A.P., 2015. Nematodes: A Threat to Sustainability of Agriculture.
922 Procedia Environ. Sci. 29, 215–216. https://doi.org/10.1016/j.proenv.2015.07.270
923 Sivasubramaniam, N., Hariharan, G., Zakeel, M.C.M., 2020. Sustainable Management of
924 Plant-Parasitic Nematodes: An Overview from Conventional Practices to Modern
925 Techniques, in: Ansari, R.A., Rizvi, R., Mahmood, I. (Eds.), Management of
926 Phytonematodes: Recent Advances and Future Challenges. Springer, Singapore, pp.
927 353–399.
928 Subedi, S., Thapa, B., Shrestha, J., 2020. Root-knot nematode (Meloidogyne incognita) and
929 its management: a review. J. Agric. Nat. Resour. 3, 21–31.
930 https://doi.org/10.3126/janr.v3i2.32298
931 Sun, H., Jiang, S., Jiang, C., Wu, C., Gao, M., Wang, Q., 2021. A review of root exudates and
932 rhizosphere microbiome for crop production. Environ. Sci. Pollut. Res. 28, 54497–
933 54510. https://doi.org/10.1007/s11356-021-15838-7
934 Takagi, H., 2020. Garlic *Allium sativum* L., in: Onions and Allied Crops. CRC Press, Boca
935 Raton, pp. 109–146. https://doi.org/https://doi.org/10.1201/9780429355752
936 Torto, B., Kirwa, H., Kihika, R., Murungi, L.K., 2018. Strategies for the Manipulation of
937 Root Knot Nematode Behavior with Natural Products in Small Scale Farming Systems.
938 ACS Symp. Ser. 1294, 114–126. https://doi.org/10.1021/bk-2018-1294.ch009
939 Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems
940 in acid rain-treated bean plants. Plant Sci. 151, 59–66. https://doi.org/10.1016/S0168-
941 9452(99)00197-1
942 Viaene, N., Coyne, D.L., Davies, K.G., 2013. Biological and cultural management, in: Perry,
943 R.N., Moens, M. (Eds.), Plant Nematology. CABI, Wallingford, pp. 383–410.
944 Vives-Peris, V., Ollas, C. de, Gómez-Cadenas, A., Pérez-Clemente, R.M., 2020. Root
945 exudates: from plant to rhizosphere and beyond. Plant Cell Rep. 39, 3–17.
946 https://doi.org/10.1007/s00299-019-02447-5
947 Wurr, D.C.E., Fellows, J.R., 1991. The influence of solar radiation and temperature on the
948 head weight of crisp lettuce The influence of solar radiation and temperature on the head
949 weight of crisp lettuce. J. Hortic. Sci. 66, 183–190.
950 https://doi.org/10.1080/00221589.1991.11516143
951

Supplementary Material

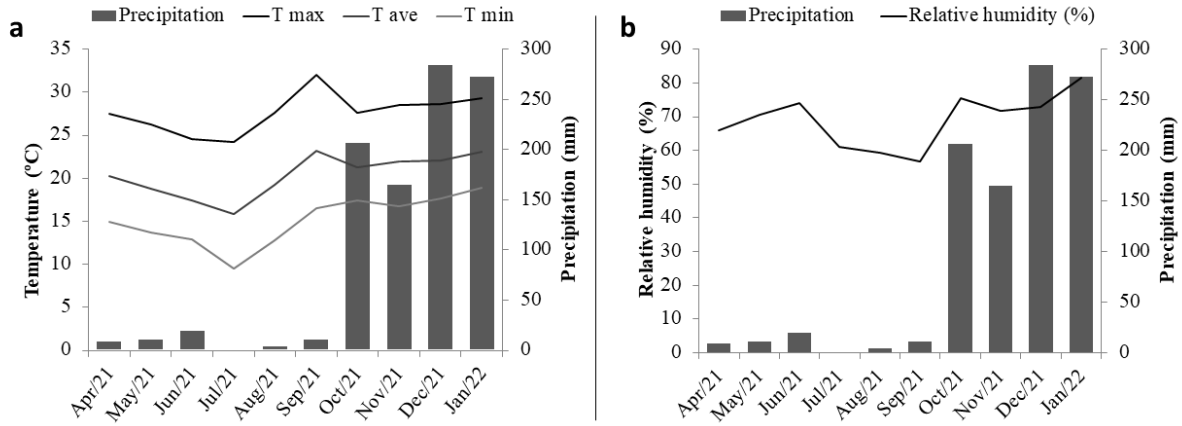

Fig. S1. Weather conditions in the region during the period in which the experiment was carried out (from April 15, 2021 to January 11, 2022). **a**) precipitation, maximum, average and minimum temperature, and **b**) precipitation and relative air humidity. Information obtained from meteorological station (code 83687) in the city of Lavras, MG. T max: maximum temperature; T ave: average temperature; T min: minimum temperature.

Table S1. Deviance analysis of the Poisson mixed-effects model for the J2 count in the soil. Result of the complete model, and the simplified model involving only the relevant fixed effects.

Model	Variation factor	Df[c]	Sum Sq	Mean Sq	F value
Complete	Tr[a]	6	32.059	5.3432	5.3432
	Time	3	5.243	1.7478	1.7478
	Tr:Time	18	38.025	2.1125	2.1125
Simplified	Lettuce	1	4.6226	4.6226	4.6226
	Garlic	1	2.2606	2.2606	2.2606
	Bsubt[b]	**1**	**14.3084**	**14.3084**	**14.3084**
	Time	3	5.3305	1.7768	1.7768
	Lettuce: Garlic	**1**	**7.1941**	**7.1941**	**7.1941**
	Garlic:Bsubt	1	3.5739	3.5739	3.5739
	Garlic:Time	**3**	**28.6792**	**9.5597**	**9.5597**
	Lettuce: Garlic:Bsubt	1	1.0782	1.0782	1.0782

[a]Treatments; [b]*Bacillus subtilis* BV09; [c]degrees of freedom.

Table S2. Comparing models for J2 count in the soil by the Chi-square test.

Modelo	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Simplified	15	353.08	393.86	-161.54	323.08			
Complete	30	372.47	454.03	-156.24	312.47	10.606	15	0.78

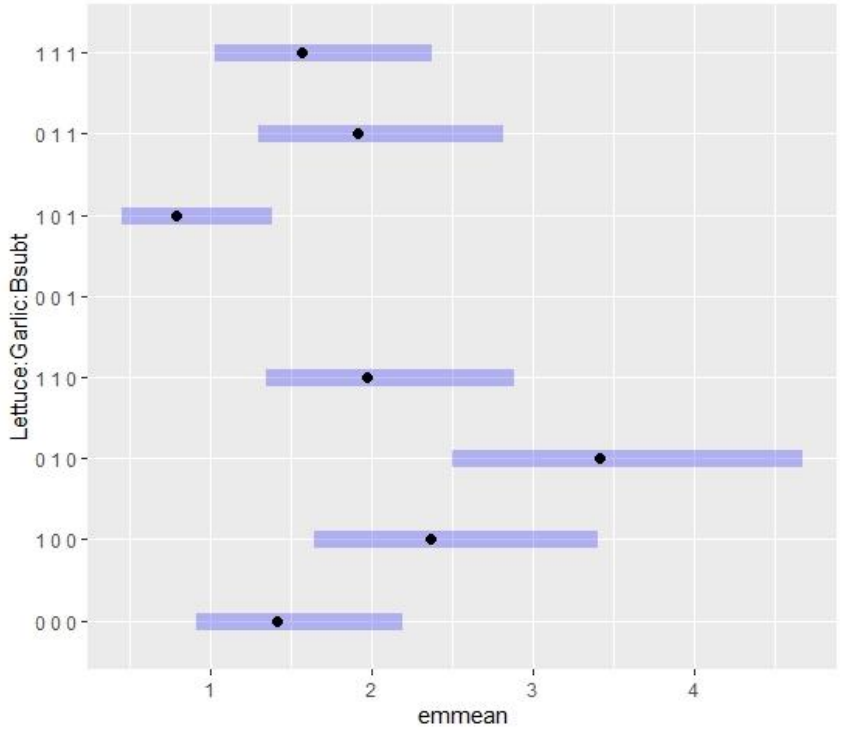

Fig. S2. Estimation of the marginal means of *M. incognita* J2 counts in 2 cm³ of soil observed in the different treatments, based on the more parsimonious Poisson mixed model. Bsubt: *B. subtilis* BV09; Emmean: estimation of marginal means.

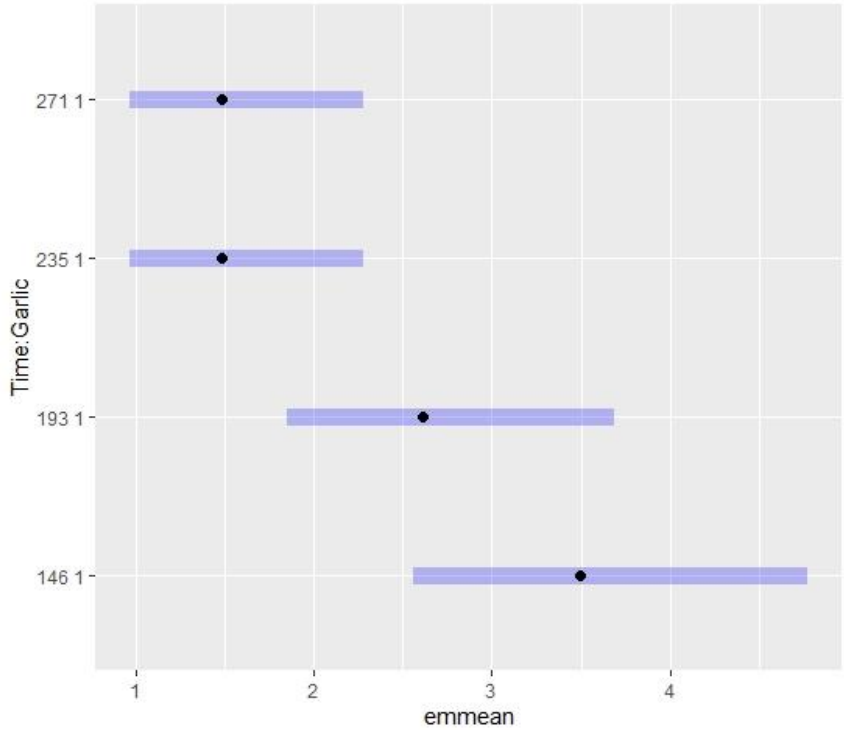

Fig. S3. Estimation of marginal means of *M. incognita* J2 counts in 2 cm³ of soil, observed at 146 days after planting (DAP) and during the evaluation of garlic residual effects at 193, 235, and 271 DAP, based on the more parsimonious Poisson mixed model. Bsubt: *B. subtilis* BV09; Emmean: estimation of marginal means.

Table S3. Summary of estimates for fixed effects from each source of variation on J2 count in the soil.

Parameter	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	0.3427	0.2242	1.5285	0.1264
Lettuce	0.5183	0.2524	2.0539	0.0400*
Garlic	0.8853	0.2457	3.6032	0.0003***
Bsubt[a]	**-1.0983**	**0.3083**	**-3.5622**	**0.0004*** **
Time	0.7877	0.2348	3.3549	0.0008***
Time²	0.2069	0.2443	0.8469	0.3970
Time³	-0.3492	0.2534	-1.3783	0.1681
Lettuce: Garlic	**-1.0694**	**0.3316**	**-3.2249**	**0.0013****
Garlic:Bsubt	0.5174	0.3771	1.3718	0.1701
Garlic:Time	**-1.4901**	**0.2895**	**-5.1463**	**<0.0001*** **
Garlic:Time²	-0.0602	0.2992	-0.2011	0.8406
Garlic:Time³	0.5363	0.3086	1.7381	0.0822
Lettuce: Garlic:Bsubt	0.3503	0.3369	1.0398	0.2984

[a]*Bacillus subtilis* BV09. Signif. codes: * p<0.05, ** p<0.01, *** p<0.001.

Table S4. Deviance analysis of the Poisson mixed-effects model for eggs count in lettuce roots. Result of the complete model, and the simplified model involving only the relevant fixed effects.

Model	Variation factor	Df[d]	Sum Sq	Mean Sq	F value
Complete	Fresh root mass	1	201.7	201.696	201.696
	Tr[a]	6	18.06	3.01	3.009
	t[b]	1	170.86	170.862	170.862
	t²	1	214.5	214.505	214.505
	t³	1	3.6	3.596	3.596
	Tr:t	6	363.34	60.557	60.557
	Tr:t²	6	136.85	22.809	22.809
Simplified	**Fresh root mass**	**1**	**182.34**	**182.34**	**182.34**
	Lettuce	1	4.18	4.18	4.1803
	Garlic	1	2.1	2.1	2.0987
	Bsubt[c]	1	0.52	0.52	0.5249
	t	**1**	**207.63**	**207.63**	**207.627**
	t²	**1**	**244.79**	**244.79**	**244.794**
	Lettuce:Garlic	1	4.75	4.75	4.7488
	Lettuce:Bsubt	1	0.02	0.02	0.024
	Garlic:Bsubt	1	4.58	4.58	4.5777
	Lettuce:t	1	8.09	8.09	8.0909
	Lettuce:t²	**1**	**70.97**	**70.97**	**70.9705**
	Garlic:t	**1**	**387.7**	**387.7**	**387.704**
	Garlic:t²	1	7.45	7.45	7.4514
	Garlic:t³	2	47.72	23.86	23.8622
	Bsubt:t	1	0.85	0.85	0.8495
	Bsubt:t²	1	12.03	12.03	12.0274
	Bsubt:t³	1	0.22	0.22	0.2201
	Lettuce:Bsubt:t	1	0.03	0.03	0.0287
	Lettuce:Bsubt:t²	1	0.28	0.28	0.281

[a]Treatments; [b]continuous time; [c]*Bacillus subtilis* BV09; [d]degrees of freedom.

Table S5. Comparing models for eggs count in lettuce roots by the Chi-square test.

Model	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
Simplified	25	1383.7	1448.8	-666.85	1333.7			
Complete	27	1385.6	1455.9	-665.8	1331.6	2.1016	2	0.3497

Table S6. Summary of estimates for fixed effects from each source of variation on eggs count in lettuce roots.

Parameter	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	3.8749	0.3270	11.849	< 2.00E-16***
Fresh root mass	**-0.3005**	**0.0439**	**-6.854**	**7.18E-12*** **
Lettuce	0.4433	0.3007	1.474	0.1405
Garlic	1.4554	0.2989	4.869	1.12E-06***
Bsubt[a]	-1.0624	0.5142	-2.066	0.0388*
t[b]	**2.1262**	**0.1160**	**18.329**	**< 2.00E-16*** **
t^2	**-1.7909**	**0.1194**	**-14.999**	**< 2.00E-16*** **
Lettuce:Garlic	-1.4641	0.4185	-3.499	0.0005***
Lettuce:Bsubt	0.4879	0.4197	1.162	0.2451
Garlic:Bsubt	0.8249	0.4199	1.964	0.0495*
Lettuce:t	-0.9874	0.0906	-10.897	< 2.00E-16***
Lettuce:t^2	**0.8428**	**0.0963**	**8.751**	**< 2.00E-16*** **
Garlic:t	**-1.5724**	**0.1135**	**-13.86**	**< 2.00E-16*** **
Garlic:t^2	-0.0162	0.0828	-0.195	0.8453
Garlic0:t^3	-0.0851	0.0839	-1.014	0.3106
Garlic:t^3	0.4279	0.0748	5.722	1.05E-08***
Bsubt:t	0.1341	0.1093	1.227	0.2199
Bsubt:t^2	-0.2576	0.1386	-1.859	0.0630
Bsubt:t^3	-0.0238	0.0967	-0.246	0.8057
Lettuce:Bsubt:t	-0.0338	0.1307	-0.258	0.7962
Lettuce:Bsubt:t^2	0.0754	0.1421	0.530	0.5959

[a]*Bacillus subtilis* BV09; [b]continuous time. Signif. codes: * p<0.05, ** p<0.01, *** p<0.001.

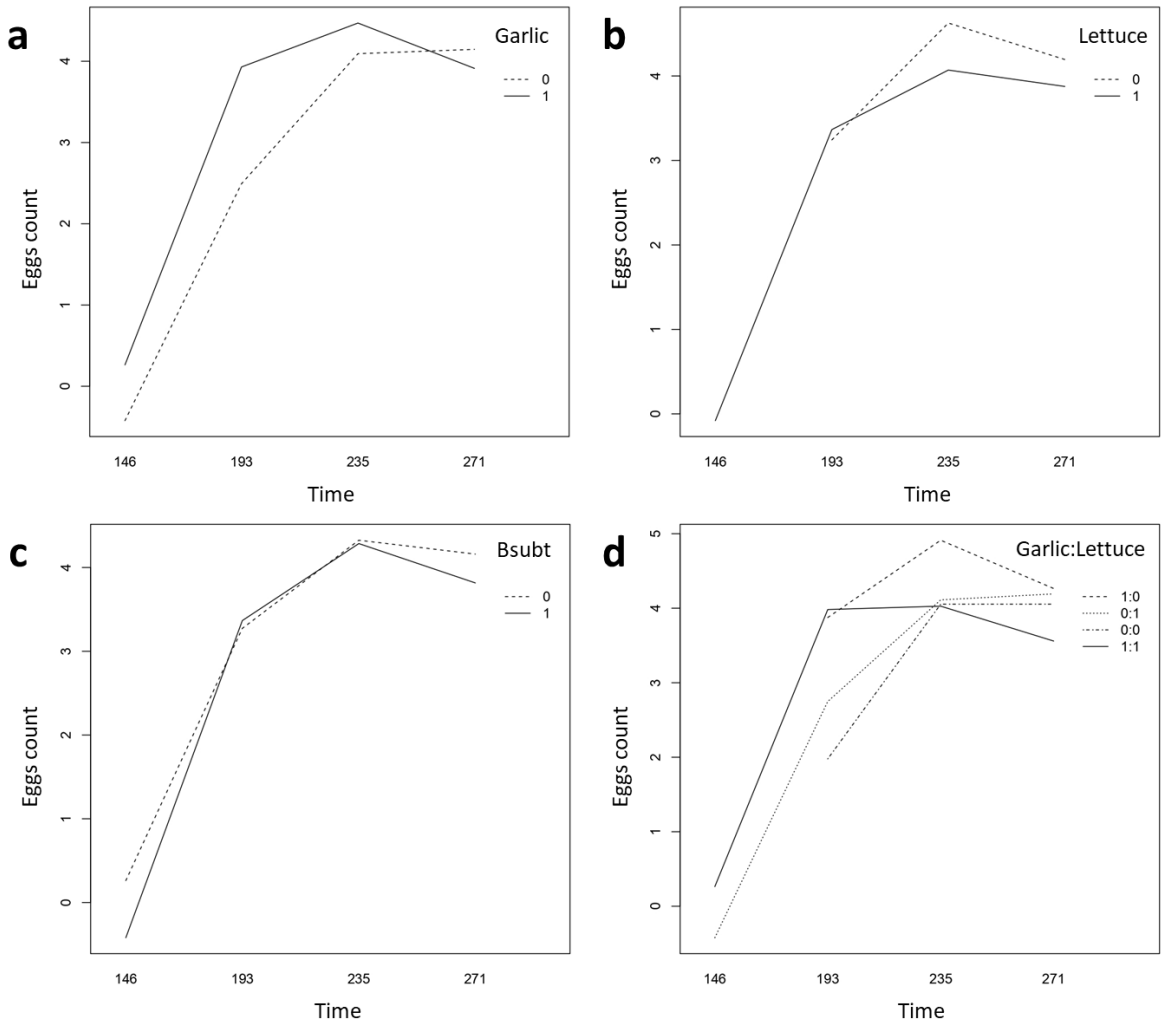

Fig. S4. Estimation of marginal means of *M. incognita* eggs count in lettuce roots over time, based on the more parsimonious Poisson mixed-effects model. This figure presents the marginal means for the factors **a**) garlic, **b**) lettuce and **c**) Bsubt, as well as for the **d**) interaction of garlic and lettuce. In graphs subtitles: 0 = presence and 1 = absence of the factors.

Table S7. Analysis of variance for the galls index in lettuce roots.

Variation factor	Df[b]	Sum Sq	Mean Sq	F value	p value
Time	**3**	**11.259**	**3.753**	**56.721**	**0.0174***
Lettuce	1	0.016	0.016	0.243	0.6221
Garlic	**1**	**1.304**	**1.304**	**19.710**	**1.25E-05*****
Bsubt[a]	**1**	**0.284**	**0.284**	**4.295**	**0.0390***
Time:Lettuce	2	0.009	0.005	0.070	0.9329
Time:Garlic	**3**	**1.041**	**0.347**	**5.247**	**0.0015****
Lettuce:Garlic	**1**	**1.731**	**1.731**	**26.155**	**5.47E-07*****
Time:Bsubt	3	0.362	0.121	1.823	0.1429
Lettuce:Bsubt	1	0.147	0.147	2.216	0.1376
Garlic:Bsubt	1	0.198	0.198	2.992	0.0847
Time:Lettuce:Garlic	2	0.224	0.112	1.694	0.1854
Time:Lettuce:Bsubt	2	0.139	0.069	1.048	0.3517
Time:Garlic:Bsubt	3	0.471	0.157	2.373	0.0703

[a]*Bacillus subtilis* BV09; [b]degrees of freedom. Signif. codes: * p<0.05, ** p<0.01, *** p<0.001.

Table S8. Summary of estimates for fixed effects from each source of variation on galls index in lettuce roots.

Parameter	Estimate	Std.Error	t value	Pr(>|t|)
Intercept	1.753	0.127	13.805	< 2.00E-16***
Time	-0.072	0.322	-0.223	0.8240
Time²	0.282	0.244	1.153	0.2498
Time³	-0.015	0.092	-0.161	0.8788
Lettuce	-0.035	0.126	-0.280	0.7797
Garlic	0.118	0.171	0.687	0.4926
Bsubt[a]	**-0.434**	**0.185**	**-2.350**	**0.0194***
Time:Lettuce	0.592	0.332	1.784	0.0753
Time²:Lettuce	-0.531	0.252	-2.108	0.0358*
Time:Garlic	0.447	0.455	0.981	0.3271
Time²:Garlic	-0.493	0.342	-1.439	0.1511
Time³:Garlic	0.089	0.104	0.856	0.3928
Lettuce:Garlic	-0.173	0.178	-0.974	0.3306
Time:Bsubt	0.651	0.479	1.359	0.1750
Time²:Bsubt	-0.203	0.370	-0.549	0.5833
Time³:Bsubt	-0.191	0.104	-1.846	0.0658
Lettuce:Bsubt	0.346	0.178	1.939	0.0534
Garlic:Bsubt	0.120	0.070	1.730	0.0847
Time:Lettuce:Garlic	-0.631	0.470	-1.343	0.1803
Time²:Lettuce:Garlic	0.632	0.356	1.775	0.0769
Time:Lettuce:Bsubt	-0.677	0.470	-1.441	0.1505
Time²:Lettuce:Bsubt	0.431	0.357	1.209	0.2277
Time:Garlic:Bsubt	-0.076	0.131	-0.582	0.5608
Time²:Garlic:Bsubt	-0.262	0.139	-1.886	0.0602
Time³:Garlic:Bsubt	0.269	0.147	1.837	0.0672

[a]*Bacillus subtilis* BV09. Signif. codes: * p<0.05, ** p<0.01, *** p<0.001.

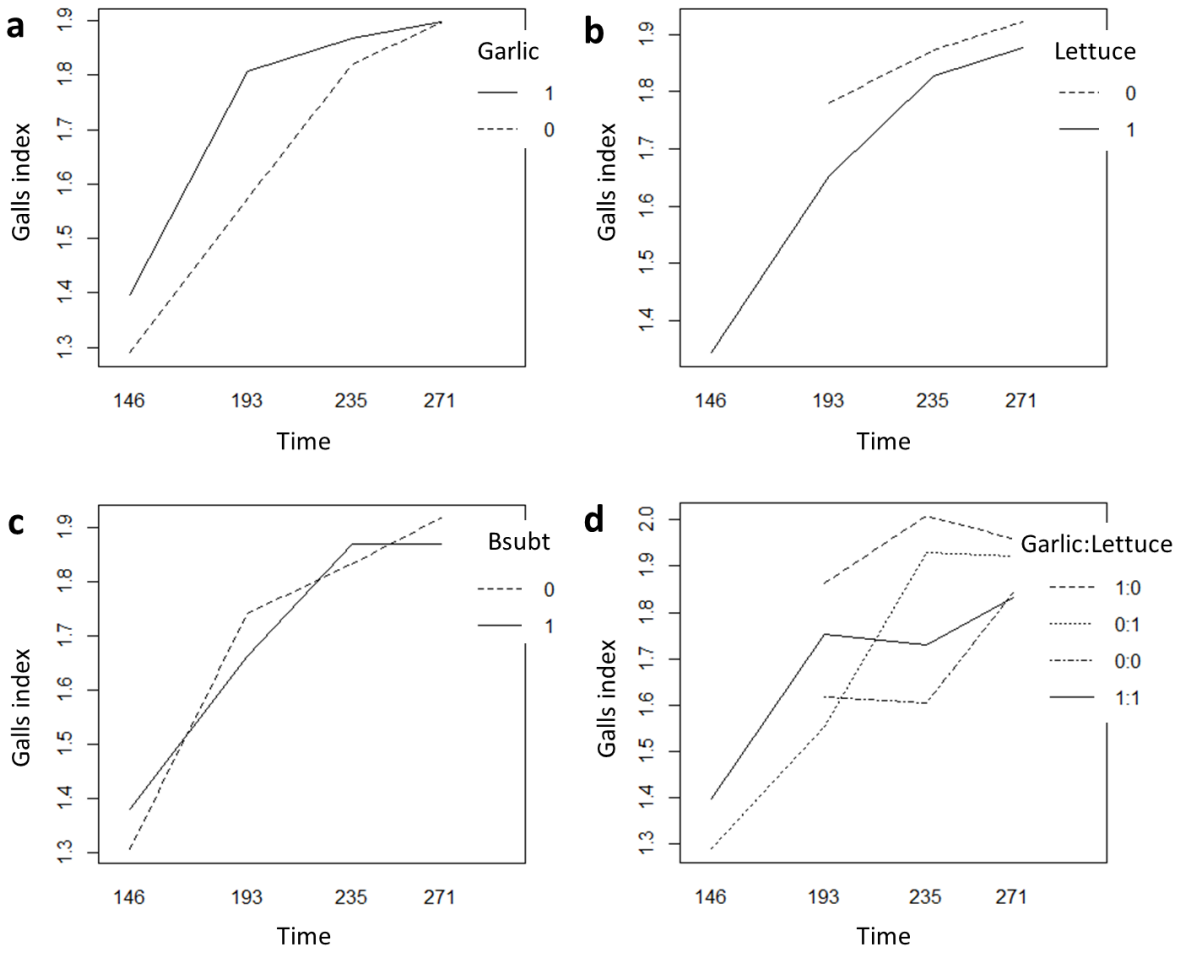

Fig. S5. Estimation of marginal means of *M. incognita* galls in lettuce roots over time, based on the linear model for the randomized complete block design. This figure presents the marginal means for the factors **a**) garlic, **b**) lettuce and **c**) Bsubt, as well as for the **d**) interaction of garlic and lettuce. In graphs subtitles: 0 = presence and 1 = absence of the factors.

Table S9. Analysis of variance for the production per lettuce plant over time.

Variation factor	Df[b]	Sum Sq	Mean Sq	F value	p value
Time	**5**	**49.262**	**9.853**	**199.888**	**0.005****
Lettuce	1	0.016	0.017	0.334	0.565
Garlic	1	0.026	0.026	0.525	0.470
Bsubt[a]	1	0.007	0.007	0.147	0.702
Time:Lettuce	2	0.296	0.148	3.005	0.054
Time:Garlic	**5**	**1.892**	**0.378**	**7.676**	**<0.001*****
Lettuce:Garlic	1	0.015	0.015	0.296	0.588
Time:Bsubt	5	0.083	0.017	0.339	0.888
Lettuce:Bsubt	1	0.054	0.054	1.092	0.299
Garlic:Bsubt	1	0.016	0.016	0.320	0.573
Time:Lettuce:Garlic	**2**	**0.332**	**0.166**	**3.363**	**0.039***
Time:Lettuce:Bsubt	2	0.096	0.048	0.972	0.382
Time:Garlic:Bsubt	5	0.169	0.034	0.687	0.634

[a]*Bacillus subtilis* BV09; [b]degrees of freedom. Signif. codes: * p<0.05, ** p<0.01, *** p<0.001.

Table S10. Summary of estimates for fixed effects from each source of variation on the production per lettuce plant over time.

Parameter	Estimate	Std.Error	t value	Pr(>|t|)
Intercept	3.5411	0.8846	4.0030	0.0001***
Time	4.1654	2.4564	1.6960	0.0931
Time²	**-2.9533**	**1.1696**	**-2.5250**	**0.0132***
Time³	**-0.5237**	**0.1397**	**-3.7480**	**0.0176***
Time⁴	**-0.6123**	**0.1140**	**-5.3700**	**0.0000*****
Time⁵	-0.0519	0.1129	-0.4600	0.6480
Lettuce	1.9061	0.8857	2.1520	0.0339*
Garlic	1.9392	1.2510	1.5500	0.1243
Bsubt[a]	-0.8651	1.2543	-0.6900	0.4920
Time:Lettuce	-4.9622	2.4572	-2.0190	0.0462*
Time²:Lettuce	1.8783	1.1748	1.5990	0.1131
Time:Garlic	-5.1853	3.4715	-1.4940	0.1385
Time²:Garlic	2.3977	1.6540	1.4500	0.1503
Time³:Garlic	-0.1647	0.1570	-1.0490	0.2968
Time⁴:Garlic	**-0.3259**	**0.1570**	**-2.0760**	**0.0405***
Time⁵:Garlic	**0.4528**	**0.1570**	**2.8840**	**0.0048****
Lettuce:Garlic	-2.0046	1.2526	-1.6000	0.1127
Time:Bsubt	2.7822	3.4786	0.8000	0.4258
Time²:Bsubt	-0.8025	1.6688	-0.4810	0.6317
Time³:Bsubt	0.0800	0.1570	0.5090	0.6116
Time⁴:Bsubt	-0.0872	0.1570	-0.5560	0.5798
Time⁵:Bsubt	-0.0009	0.1570	-0.0060	0.9955
Lettuce:Bsubt	0.8439	1.2526	0.6740	0.5021
Garlic:Bsubt	-0.0513	0.0906	-0.5650	0.5730
Time:Lettuce:Garlic	5.3962	3.4751	1.5530	0.1237
Time²:Lettuce:Garlic	-1.8812	1.6614	-1.1320	0.2603
Time:Lettuce:Bsubt	-2.7271	3.4751	-0.7850	0.4345
Time²:Lettuce:Bsubt	0.9184	1.6614	0.5530	0.5817
Time:Garlic:Bsubt	-0.2490	0.2220	-1.1220	0.2647
Time²:Garlic:Bsubt	-0.2241	0.2220	-1.0090	0.3154
Time³:Garlic:Bsubt	-0.0561	0.2220	-0.2530	0.8010
Time⁴:Garlic:Bsubt	0.2274	0.2220	1.0240	0.3083
Time⁵:Garlic:Bsubt	-0.0472	0.2220	-0.2130	0.8321

[a]*Bacillus subtilis* BV09. Signif. codes: * p<0.05, ** p<0.01, *** p<0.001.

Table S11. Analysis of variance for the production per garlic plant at 146 days after planting.

	Df[b]	F value	
Blocks	1	0.001	
Treatments	3	12.377	**
Lettuce	**1**	**26.4082**	****
Bsubt[a]	1	2.6199	
Lettuce:Bsubt	**1**	**8.1034**	**
Residual Degrees of freedom		7	
Residual Mean Square		0.11609	

[a]*Bacillus subtilis* BV09; [b] degrees of freedom. Signif. codes: * p<0.05, ** p<0.01, *** p<0.001.

Table S12. Marginal means for the production per lettuce plant under the influence of lettuce and garlic factors over time, based on the linear model for the randomized complete block design.

DAP[a]	Lettuce	Garlic	Mean	SE[b]	LCL[c]	UCL[d]
46	0	0	NA	NA	NA	NA
	1	0	221.6	17.58	177	277.5
	0	1	NA	NA	NA	NA
	1	1	240.7	19.09	192.3	301.4
96	0	0	NA	NA	NA	NA
	1	0	374.8	47.55	178.1	788.4
	0	1	NA	NA	NA	NA
	1	1	370.6	47.01	176.1	779.6
146	0	0	NA	NA	NA	NA
	1	**0**	**274**	**22.06**	**216.9**	**346.2**
	0	1	NA	NA	NA	NA
	1	**1**	**138**	**11.11**	**109.2**	**174.3**
193	0	0	184.9	21.19	145.8	234.5
	1	0	284.8	23.78	234	346.7
	0	1	278.4	23.24	228.7	338.9
	1	1	282.1	23.55	231.7	343.4
235	0	0	400.9	52.15	294.4	545.9
	1	0	369.6	38.34	263	519.5
	0	1	385.1	39.95	274	541.3
	1	1	362.8	37.64	258.2	509.9
271	0	0	60.6	7.01	47.6	77.2
	1	0	61.2	5.2	49.9	75.1
	0	1	66.3	5.63	54	81.4
	1	1	67.4	5.73	54.9	82.7

[a]Days after planting; [b]standard error; [c]lower confidence limits; [d]upper confidence limits.

Table S13. Averages of production per garlic plant at 146 days after planting, based on the linear model for the randomized complete block design.

Alface	Bsubt	Average	SE[b]	LCL[c]	UCL[d]
0	0	28.4	2.13	23.6	33.7
1	0	33.4	2.31	28.2	39.1
0	1	25.9	2.03	21.3	30.9
1	1	44.4	2.66	38.3	50.9

[a]*Bacillus subtilis* BV09; [b]standard error; [c]lower confidence limits; [d]upper confidence limits.

Table S14. Analysis of variance for the estimated total gross and net income from the lettuce and garlic production.

		Total gross income		Total net income	
	Df[b]	F value		F value	
Blocks	1	2.852		1.907	
Treatments	6	22.058	***	13.333	***
Lettuce	**1**	**106.605**	*******	**74.1**	*******
Garlic	1	0.0031		0.1271	
Bsubt[a]	1	0.2653		0.0381	
Lettuce:Garlic	**1**	**25.3999**	*******	**5.7222**	*****
Lettuce:Bsubt	1	0.0428		0.0074	
Garlic:Bsubt	1	0.0302		0.0044	
Residual Degrees of freedom		16		16	
Residual Mean Square		3.81×10^{9}		4.68×10^{9}	

[a]*Bacillus subtilis* BV09; [b]degrees of freedom. Signif. codes: * p<0.05, ** p<0.01, *** p<0.001.

Table S15. Averages of total gross and net income observed in the different treatments, based on the linear model for the randomized complete block design.

			Total gross income (x 1,000 R$)				Total net income (x 1,000 R$)			
Lettuce	**Garlic**	**Bsubt[a]**	**Average**	**SE[b]**	**LCL[c]**	**UCL[d]**	**Average**	**SE**	**LCL**	**UCL**
1	0	0	795.715	30.855	730.305	861.125	615.715	34.215	543.183	688.247
1	0	1	774.967	30.855	709.557	840.377	594.967	34.215	522.435	667.499
0	1	0	542.134	35.890	466.052	618.217	374.773	39.797	290.407	459.139
0	1	1	528.884	35.890	452.801	604.966	346.177	39.797	261.811	430.543
1	1	0	693.073	35.890	616.991	769.156	554.277	39.797	469.911	638.643
1	1	1	660.736	35.890	584.653	736.818	528.637	39.797	444.270	613.003
0	0	0	384.099	30.855	318.689	449.509	294.099	34.215	221.567	366.631

[a]*Bacillus subtilis* BV09; [b]standard error; [c]lower confidence limits; [d]upper confidence limits.

Table S16. Analysis of variance for the superoxide dismutase (SOD), ascorbate peroxidase (APX) and lipid peroxidation in lettuce leaves.

		SOD		APX		Lipid peroxidation	
	Df[b]	F value		F value		F value	
Blocks	1	4.1708		0.4437		4.3483	*
Time	1	3.0096		**5.5338**	*****	**110.59**	*******
Garlic	1	0.7335		0.8064		**16.663**	*******
Bsubt[a]	1	3.8198		0.5495		0.2835	
Time:Garlic	1	**5.9416**	*****	0.2315		**24.259**	*******
Time:Bsubt	1	1.914		0.0475		1.6556	
Garlic:Bsubt	1	0.3515		0.1751		0.3862	
Time:Garlic:Bsubt	1	0.1394		0.2138		**25.14**	*******
Residual Degrees of freedom		23		23		23	
Residual Mean Square		0.0065		1.2723		234.8	

[a]*Bacillus subtilis* BV09; [b]degrees of freedom. Signif. codes: * p<0.05, ** p<0.01, *** p<0.001.

Table S17. Analysis of variance for the hydrogen peroxide (H_2O_2) and lipid peroxidation in garlic leaves.

		H_2O_2		Lipid peroxidation	
	Df [b]	F value		F value	
Blocks	1	6.305	*	0.1518	
Time	1	**40.0391**	***	**25.4775**	***
Lettuce	1	0.6089		**18.8594**	***
Bsubt [a]	1	1.586		1.2801	
Time:Lettuce	1	0.2221		1.2229	
Time:Bsubt	1	0.1003		**16.0034**	***
Lettuce:Bsubt	1	0.0908		**7.645**	*
Time:Lettuce:Bsubt	1	0.1677		0.0968	
Residual Degrees of freedom		23		23	
Residual Mean Square		0.05235		1014.5	

[a]*Bacillus subtilis* BV09; [b]degrees of freedom. Signif. codes: * p<0.05, ** p<0.01, *** p<0.001.

Table S18. Multivariate analysis (MANOVA) performed for garlic foliar nutrients content as affected by lettuce cultivation, *B.subtilis* BV09 application and their interaction.

	Df	**Pillai**	**approx F**	**num Df**	**den Df**	**Pr(>F)**
Lettuce	1	0.960	4.356	11	2	0.201
Bsubt[a]	1	0.678	0.383	11	2	0.882
Lettuce:Bsubt	1	0.814	0.798	11	2	0.677
Residuals	12					

[a]*Bacillus subtilis* BV09.

Table S19. Analysis of variance for the nitrogen, phosphorus and potassium content in lettuce leaves.

Variation factor	**Df** [b]	**Nitrogen (N)**			**Phosphorus (P)**			**Potassium (K)**		
		F	**p**		**F**	**p**		**F**	**p**	
Lettuce	1	22.314	0.0005	***	5.765	0.033	*	7.185	0.020	*
Bsubt [a]	1	0.209	0.656		0.471	0.506		0.090	0.769	
Lettuce:Bsubt	1	0.399	0.540		0.471	0.506		0.053	0.821	
Residual Degrees of freedom		12			12			12		
Residual Mean Square		1.319			0.021			4.682		

[a]*Bacillus subtilis* BV09.

Table S20. Multivariate analysis (MANOVA) performed for lettuce foliar nutrients content as affected by garlic cultivation, *B.subtilis* BV09 application and their interaction.

	Df	**Pillai**	**approx F**	**num Df**	**den Df**	**Pr(>F)**
Garlic	**1**	**0.998**	**84.082**	**11**	**2**	**0.012 ***
Bsubt[a]	1	0.976	7.429	11	2	0.125
Garlic:Bsubt	**1**	**0.998**	**98.325**	**11**	**2**	**0.010 ***
Residuals	12					

[a]*Bacillus subtilis* BV09.

Table S21. Analysis of variance for the phosphorus (P) content in lettuce leaves.

Variation factor	Df	Sum Sq	Mean Sq	F	p
Garlic	**1**	**1.96**	**1.96**	**8.415**	**0.0133 ***
Bsubt[a]	1	0.16	0.16	0.6869	0.4234
Garlic:Bsubt	1	0.0625	0.0625	0.2683	0.6139
Residuals	12	2.795	0.23292		

[a]*Bacillus subtilis* BV09.

Versão preliminar submetida ao periódico: Plant Physiology and Biochemistry

Bacillus subtilis BV09 affecting root exudates metabolomics and *Meloidogyne incognita* chemotactic response

ABSTRACT

Root exudates mediate plant interactions in the environment, and they can be affected by physical, chemical, and biological factors. Biocontrol agents can affect root exudates enhancing plant tolerance to pathogens. Lettuce (*Lactuca sativa*) is a host plant to root-knot nematode (*Meloidogyne incognita*), while garlic (*Allium sativum*) is an antagonistic plant. In this paper, the effects of *Bacillus subtilis* BV09 application in the chemotactic response of *M. incognita* to lettuce and garlic root exudates, as well as in the metabolomics of these root exudates were evaluated. Lettuce and garlic root exudates were collected from both plants with and without the application of *B. subtilis* BV09 (commercial product: Biobaci®) in their roots. A chemotaxis assay was performed with the root exudates, as well as with *B. subtilis* BV09 suspension. Metabolomics analysis was performed with these samples. Lettuce root exudates were attractive to *M. incognita* J2, but with the presence of *B. subtilis* BV09 became repellent. Garlic root exudates were repellent to *M. incognita* and became attractive with the presence of *B. subtilis* BV09. In the metabolomics analysis, the application of *B. subtilis* BV09 in lettuce and garlic roots affected qualitative and quantitatively their roots exudates as compared to plant exudates from lettuce and garlic without *B. subtilis* BV09 application. For example, the number of amino acids and peptides was higher with the inoculation of *B. subtilis* BV09. Thus, it was concluded that the application of *B. subtilis* BV09 in lettuce and garlic roots affected *M. incognita* chemotactic response to their root exudates since modified its chemical composition.

Palavras-chave: *Allium sativum*, biological control, chemotaxis, *Lactuca sativa*, root-knot nematode.

1. INTRODUCTION

Root exudates are a mixture of substances from primary and secondary metabolism released by plant roots into the surrounding space, which can be composed of sugars, amino acids, peptides, enzymes, vitamins, organic acids, nucleotides, phenolics, and other secondary metabolites, playing important roles in subterranean ecology (Haichar et al., 2014; Rasmann et al., 2012; Rovira, 1969; Vives-Peris et al., 2020). The metabolites released into the soil are important in mediating interactions between plants in the environment, as well as mediating plant interaction with pests and diseases, such as herbivorous insects, plant-parasitic nematodes (PPN), fungi and bacteria (Delory et al., 2016; Vives-Peris et al., 2020).

Most plant-parasitic nematodes are found in soil ecosystems and use chemosensory perception to perceive their surroundings and guide their movement to find hosts (Sikder and Vestergård, 2020). Host roots release signals in the soil, generating chemical stimuli for PPN, which diffuse through the soil (Ali et al., 2011; Siddique et al., 2022).

In addition to the presence of signaling molecules, root exudates can also have antagonist molecules to PPN, i.e., negative effects on the PPN. Several bioactive plant compounds with this action are known, such as polythienyls, isothiocyanates, glucosinolates, cyanogenic glycosides, polyacetylenes, alkaloids, lipids, terpenoids, sesquiterpenoids, diterpenoids, limonoid triterpenes, quassinoids, steroids, triterpenoids, simple and complex phenolic compounds, flavonoids, saponins, tannins, essential oils, fatty acids, among others (Chitwood, 2002; D'Addabbo et al., 2014; Dong et al., 2018).

Root exudates can undergo positive or negative changes in their composition, as well as changes in each metabolite's quantity, due to physical, chemical, and biological factors, as light intensity, extreme temperatures, water availability, phytohormones, nutrient concentration, plant age, allelopathy, and plant pathogens (Vives-Peris et al., 2020).

Meloidogyne incognita, known as root-knot nematode (RKN), is a cosmopolitan and generalist species of plant-parasitic nematode well adapted to the climatic conditions of tropical and subtropical regions and economically important for several crops (Sikora and Fernandez, 2005). Lettuce (*Lactuca sativa* L.) is among the host plants and root-knot nematode (*Meloidogyne* spp.) attack can cause losses in more than 80% of its production (Charchar and Moita, 2005). While garlic (*Allium sativum* L.) is an antagonistic plant to root-knot nematode (RKN), showing nematicide properties in its bulbs aqueous extracts and essential oil (Eder et al., 2021; Jardim et al., 2020; Kouamé, 2021).

Bacillus subtilis is a bacterium species well established as plant growth promoting rhizobacteria (PGPR) and biocontrol agent (BCA), used in the control of *Meloidogyne* spp. with efficient results (Basyony and Abo-Zaid, 2018; Cao et al., 2019). *Bacillus* spp. act in PPN control through direct modes of action, by colonizing the roots, releasing nematicide metabolites (antibiosis) and affecting plant-pathogen communication, in addition to indirect modes of action, such as induction of systemic resistance enhancing host defense mechanisms (Júnior et al., 2022; Migunova and Sasanelli, 2021). The application of PGPR as well as BCA can affect root exudation and enhance plant tolerance to abiotic and biotic stresses (Ansari et al., 2020; Moosavi, 2020). Therefore, the objective was to evaluate the metabolomics changes of lettuce and garlic root exudates when inoculated with *Bacillus subtilis* BV09 (commercial product: Biobaci®), as well as the chemotactic response of *Meloidogyne incognita* to these root exudates.

2. MATERIAL AND METHODS

2.1 Obtaining the plant-parasitic nematode

The sedentary endoparasitic nematode *Meloidogyne incognita* (Kofoid & White) Chitwood was used due to its large number of host plants and worldwide distribution

(Pinheiro et al., 2014; Sikora and Fernandez, 2005). The *M. incognita* population was previously established in tomato plants (*Solanum lycopersicum* L.) kept in a greenhouse. Eggs were extracted and collected according to the method described by Hussey and Barker (1973). To obtain second-stage juveniles (J2), the eggs were placed in an adapted Baermann funnel with available materials.

2.2 Chemotactic response of *Meloidogyne incognita* to the root exudates

To analyze the effect of the metabolites released by plant roots on the behavior of second-stage juveniles (J2) of *Meloidogyne incognita*, root exudates of lettuce and garlic plants were collected, with and without the application of a *Bacillus*-based product indicated to the control of *M. incognita* (commercial product: Biobaci®, active ingredient: *Bacillus subtilis* BV09). Then, the chemotaxis index of these samples was performed.

2.2.1 Collection of the root exudates

The root exudates collection was based on the methods described by Čepulyte et al. (2018) and Liu et al. (2019). Lettuce and garlic plants with two to three weeks of growth were used. The roots were washed and placed inside a glass flask immersed in distilled and sterilized water, leaving the aerial part out of the flask (Fig. 1, I). For lettuce plants, the roots of 20 plants were placed (around 0.2 grams of roots per flask) submerged in 4 mL of sterilized distilled water (T1). For garlic plants, the roots of five plants were placed (around 5 grams of roots per flask) submerged in 15 mL of distilled and sterilized water (T3). To evaluate the effect of Biobaci® on the root exudates of lettuce and garlic (T2 and T4), 100 µL of *B. subtilis* BV09 suspension (Biobaci®) were added to the flasks with lettuce or garlic roots submerged in distilled and sterilized water. Two controls were used, one with 100 µL of the suspension of *B. subtilis* BV09 diluted in 4 mL of distilled and sterilized water (T5), and the

other one with distilled and sterilized water (T6) (Table 1). To protect the roots from light, the flasks were wrapped in aluminum foil. Plants were placed inside a moist chamber with 98% humidity and kept in a growth room during 24 hours at 24ºC with 16 hours photoperiod (Fig. 1, I). Then, the water was collected, filtered (0.22 µm) and stored in microtubes (Čepulyte et al., 2018). The samples were frozen (-80ºC), lyophilized and kept in a freezer until they were resuspended in 20 µL (Fig. 1, II) and used in the chemotaxis assay (Fig. 1, III) or in the metabolomics analysis.

Table 1. Description of the treatments.

Treatments	Description
T1	Root exudates of lettuce plants
T2	Root exudates of lettuce plants with *B. subtilis* BV09 (Biobaci®) application
T3	Root exudates of garlic plants
T4	Root exudates of garlic plants with *B. subtilis* BV09 (Biobaci®) application
T5	Suspension of *B. subtilis* BV09 (Biobaci®)
T6	Distilled and sterilized water

2.2.2 Chemotaxis index

To observe chemotactic response of *M. incognita* to root exudates, a chemotaxis assay was performed based on the method described by Wang et al. (2021) with some modifications. Petri dishes 6 cm in diameter with 10 mL of agar–water medium (2%, 2 g/L) were used to observe the movement of the second-stage juveniles (J2) of *M. incognita*. Approximately 200 J2 were placed in the center of the Petri dish (neutral area, Fig. 1, III). A total of 10 µL of root exudates was added at the edge of the test area (+), and 10 µL of distilled water was added at the edge of the control area (-). A negative control was performed by adding only water at the edge of the test and control areas. Then, the plates were kept at room temperature in the dark. After 16 h, the number of J2 in the test and control areas was quantified. The chemotaxis index (CI) was calculated using the equation below:

$$\mathrm{CI} = \frac{(\text{number of J2 in the test area} - \text{number of J2 in the control area})}{(\text{number of J2 in the test area} + \text{number of J2 in the control area})}$$

According to Wang et al. (2019), CI values ≥0.2 were considered highly attractive, CI ≥0.1 but <0.2 as slightly attractive, CI ≥−0.1 but <0.1 as a patternless response, CI >−0.2 but <−0.1as slightly repellent, and a CI ≤−0.2 as highly repellent.

The treatments used in the chemotaxis assay corresponded to those described in Table 1, with 10 repetitions performed per treatment. This assay was performed twice, being called experiment I and experiment II.

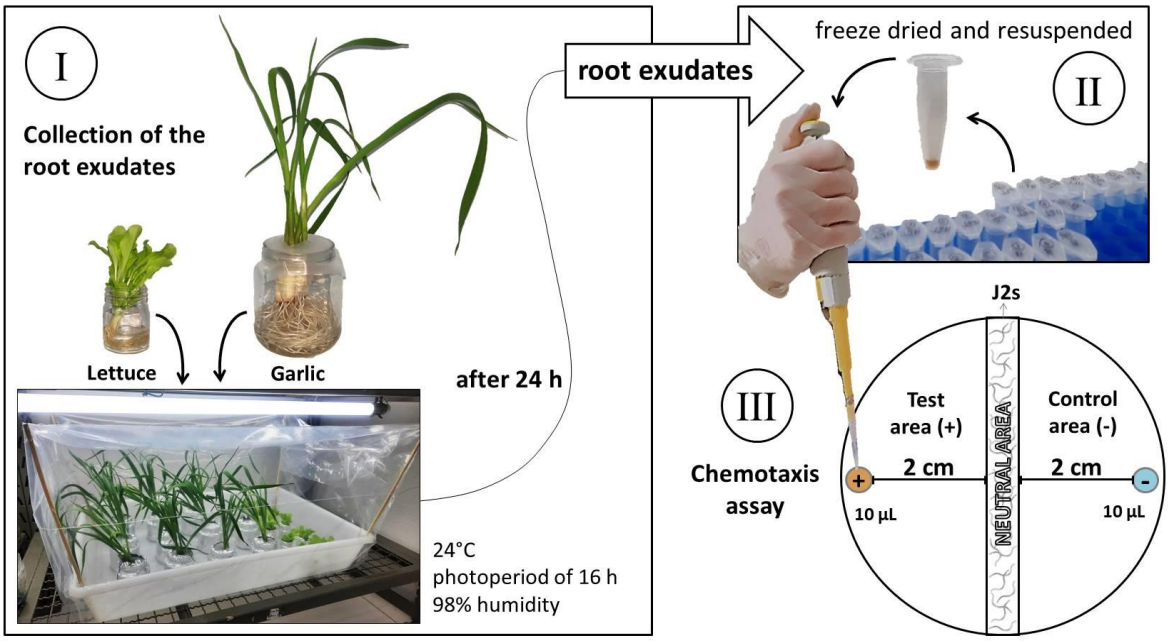

Fig. 1. Collection of the root exudates (I and II) and chemotaxis assay (III) to observe their effects on the movement of *M. incognita*. The J2 were placed in the center (neutral area) of a 6 cm diameter Petri dish, the root exudates were added to the test area (+), and distilled water was added to the control area (-). The chemotaxis assay was adapted from Wang et al. (2021).

2.3 Metabolomic HPLC-MS

2.3.1 Sample preparation

Three plastic microtubes of each sample (T1, T2, T3 and T4) were separated for analysis. The extraction was performed by adding 100 µL of a H_2O:MeOH (8:2) solution with 2 µg mL^{-1} of the internal standard p-fluoro-DL-phenylalanine ($C_9H_{10}FNO_2$, Rt 2.22 min, m/z 184.076830 [M+ H]+) added to the first microtube. The sample was homogenized in a vortex mixer for 60 seconds. Then, first microtube content was transferred to a second one of the same sample and the extraction was performed in the same way. The second microtube content was transferred to a third. The sample was homogenized in a vortex mixer for 60 seconds and placed in an ultrasonic bath for 10 minutes without heating. The samples were centrifuged at 5000 g for 10 minutes and 80 µL of the supernatant was transferred to vials with glass inserts for analysis. For the T5 sample (*B. subtilis* BV09 suspension, Biobaci®), two microtubes were used with the sample and the extraction took place in the same way as for the other samples. In addition, four extraction blanks were performed, referring to the different types of samples.

2.3.2 Chromatographic method and mass spectrometer parameters

Chromatographic analyzes were performed using a Dionex LC UltiMate 3000 liquid chromatograph coupled to a Q-Exactive Plus high-resolution mass spectrometer (Thermo Scientific, Frenton, CA, USA). The chromatographic method used in analyzes was performed using the Thermo Syncronis C_{18} column 50 x 2.1 mm, 1.7 µm of particle size. Mobile phase A was water with 0.1% (v/v) formic acid, and mobile phase B was methanol with 0.1% (v/v) formic acid. The chromatographic separation was performed in gradient elution mode at a flow rate of 0.35 mL min^{-1} as follows: 0–9 min B 5–60%, 9–13 min B 60–98%, 13–16 min B 98%, 16.1–20 min B 5%. Sample injection volume was 8 µL and the column oven temperature was 40°C.

Mass spectrometer data acquisition was performed in positive electrospray ionization mode. The ionization source conditions were: sheath gas: 45 arbitrary units (a.u.), auxiliary gas: 15 a.u., spray voltage: 3.9 kV, S-lens voltage: 60 V, capillary temperature: 320°C, source temperature: 400°C. A full ion scan was performed in the m/z range of 100-1000 with a resolution of 70,000 FWHM (full width at half maximum), Automatic Gain Control (AGC) target of 1e6 and Maximum ion injection time (IT) 100 ms, combined with data-dependent acquisition DDA Top5 type at a resolution of 17,500 FWHM, AGC target of 1e5, Maximum IT 50 ms and an isolation window of 1.2 Da.

2.3.3 Compounds annotation

Compounds annotation was performed by comparing the m/z values of the precursor ions obtained experimentally to those theoretical values calculated or available in the spectra libraries with an error smaller than 5 ppm (Table S1). Annotations were also based on similarities between the fragmentations spectra (MS/MS) obtained with data from spectral libraries. For this compounds annotation, the raw spectra (RAW) were submitted to processing in MS-DIAL v4.8 software using the following parameters: MS1 and MS2 tolerances: 0.005 and 0.05 Da, respectively; minimum peak height: 500,000; mass width: 0.05 Da; sigma window value for deconvolution: 0.4; 0.1 min and 0.005 Da tolerances for peak alignment; similarity score higher than 80% between spectra. The libraries used were Mass Bank of North America (MONA, public) and NIST MSMS 2020 (commercial).

2.4 Statistical analysis

The normality of the data was evaluated by the Shapiro–Wilk test, and the homogeneity of the variances was evaluated by the Levene test. Then, the data were subjected to analysis of variance (ANOVA) and the means were grouped by the Scott–Knott test at 5%

significance. The R software was used to perform the statistical analyses (R Core Team, 2019).

3. RESULTS

3.1 Chemotactic response of *Meloidogyne incognita* to the root exudates

The treatments had a significant effect ($p < 0.05$) on the chemotactic response of the *M. incognita* J2 (Table 3), being grouped by the Scott-Knot test in three groups: (a) the treatments that slightly attracted the J2, (b) the treatments in which the J2 showed a random response or were slightly repelled, and (c) the treatments that highly repelled the J2 (Table 4). Experiments I and II showed similar results.

Table 3. Analysis of variance for the chemotaxis index from the different treatments in the experiments I and II.

		Experiment I					Experiment II				
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**		**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	
Treatments	5	3.671	0.734	18.723	9.50E-11	***	3.671	0.734	35.417	6.91E-16	***
Residuals	54	2.118	0.039				1.120	0.021			

Table 4. Average of chemotaxis index from root exudates of lettuce and garlic, with and without the application of *B. subtilis* BV09 (Biobaci®), in the experiments I and II.

		Experiment I				Experiment II			
	Treatments	**Mean**	**SE**	**LCL**	**UCL**	**Mean**	**SE**	**LCL**	**UCL**
T1	Lettuce	0.104 a	0.063	-0.022	0.229	0.119 a	0.046	0.028	0.210
T2	Lettuce+Bsubt	-0.509 c	0.063	-0.634	-0.383	-0.532 c	0.046	-0.623	-0.441
T3	Garlic	-0.157 b	0.063	-0.283	-0.032	-0.112 b	0.046	-0.204	-0.021
T4	Garlic+Bsubt	0.162 a	0.063	0.036	0.288	0.074 a	0.046	-0.017	0.166
T5	Bsubt	-0.391 c	0.063	-0.516	-0.265	-0.440 c	0.046	-0.532	-0.349
T6	Control	-0.007 b	0.063	-0.132	0.119	-0.036 b	0.046	-0.127	0.056

Bsubt – *B. subtilis* BV09 (Biobaci®); SE – standard error; LCL – lower confidence limits; UCL – upper confidence limits. * Means followed by the same letter in the columns were grouped by the Scott-knott test ($p < 0.05$).

The *M. incognita* J2 were slightly attracted by lettuce root exudates (T1), with an average chemotaxis index (CI) between 0.1 and 0.2. While for lettuce root exudates with the

application of *B. subtilis* BV09 (T2), the *M. incognita* J2 were highly repelled, with an average CI smaller than -0.2 (Fig. 2 and Table 4).

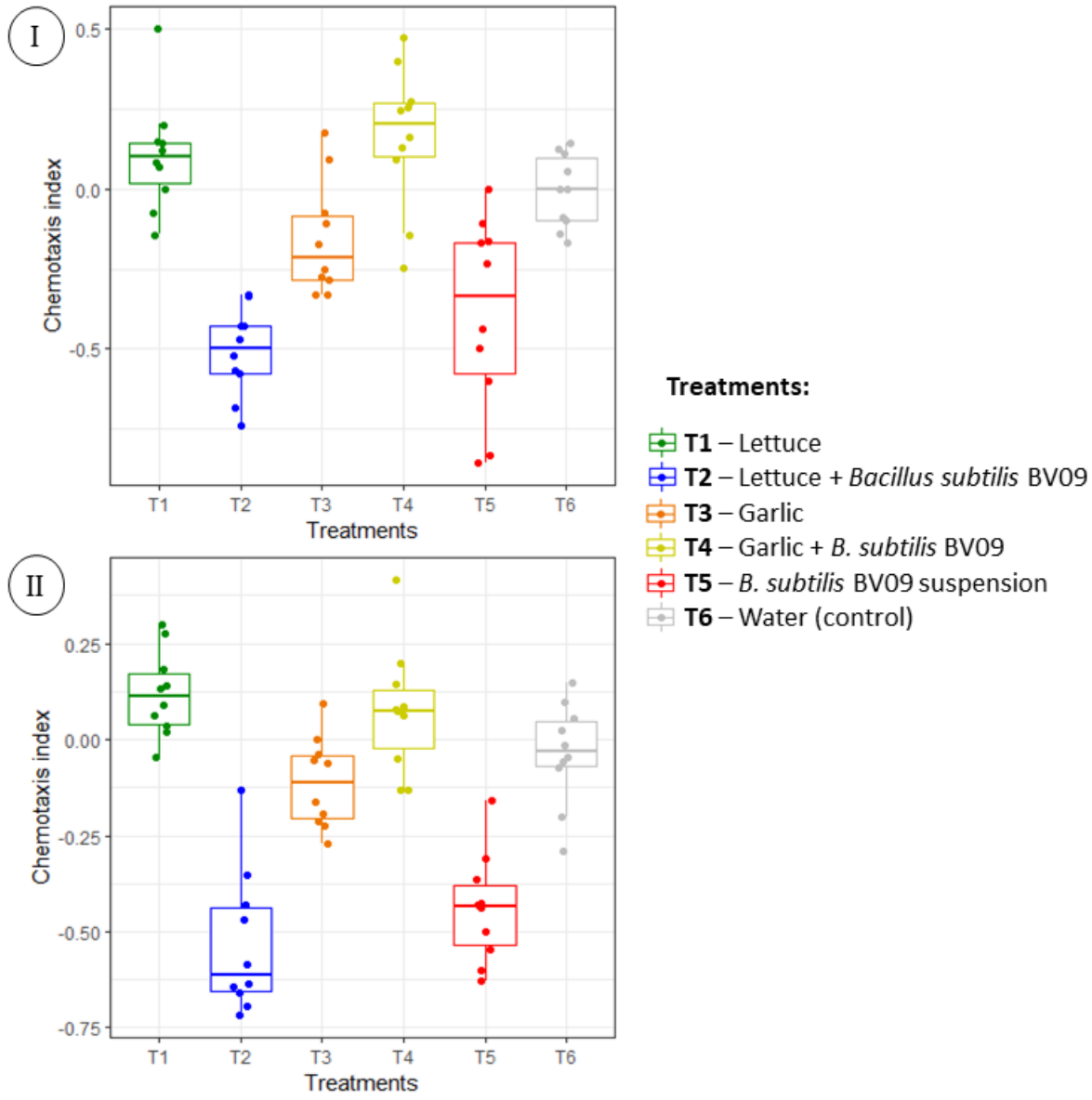

Fig. 2. Chemotaxis index (CI) of the root exudates from lettuce and garlic plants with and without the application of *B. subtilis* BV09 (Biobaci®). This Fig. presents the results of the first (I) and second (II) experiment. T1 (green) = Root exudates of lettuce plants; T2 (blue) = Root exudates of lettuce plants with the application of *B. subtilis* BV09; T3 (orange) = Root exudates of garlic plants; T4 (yellow) = Root exudates of garlic plants with the application of *B. subtilis* BV09; T5 (red) = Suspension of *B. subtilis* BV09; T6 (grey) = Distilled and sterilized water.

Garlic root exudates (T3) caused slight repellency to *M. incognita* J2, with an average CI smaller than -0.1. When applying *B. subtilis* BV09 on garlic roots (T4), the root exudates stopped being repellent and started to promote a slight attractiveness to the *M. incognita* J2, with an average CI around 0.1 (Fig. 2 and Table 4).

For the controls, the *M. incognita* J2 were highly repelled by the *B. subtilis* BV09 suspension (T5), with an average CI smaller than -0.2, while they showed a random response to the distilled and sterilized water (T6), with CI values below 0.1 and above -0.1 (Fig. 2 and Table 4).

3.2 Metabolomics of plant root exudates in response to *B. subtilis* BV09 application

Metabolomics analysis was performed to identify differential metabolites in lettuce and garlic root exudates, with and without the application of *B. subtilis* BV09 (Biobaci®), once differential metabolites can reveal plants' physiological responses in the presence of *B. subtilis* BV09 and help the understanding of *M. incognita* J2 behavior. A total of 34 metabolites were annotated (Table S1), including 20 amino acids and peptides (**3-7**, **10**, **13-15**, **17-21**, **23**, **25-28** and **30**), 2 fatty acid (**29** and **31**), 2 nucleotides and nucleosides (**11** and **12**), 2 organic acids (**1** and **8**) and 2 vitamins (**9** and **16**) coming from primary metabolism, 2 alkaloids (**22** and **24**) and 1 phenolic compound (**32**) coming from secondary metabolism, 1 phytohormone and 2 other metabolites (**2** and **34**) (Table 5).

The treatments with the application of *B. subtilis* BV09 in lettuce and garlic roots (T2 and T4, respectively) presented a higher number of metabolites than the treatments with the absence of *B. subtilis* BV09 (T1 and T3). *B. subtilis* BV09 suspension also showed a high number of metabolites (Table 5).

Through the chromatograms it is possible to verify the metabolites complexity of *B. subtilis* BV09 suspension with many peaks of high and low relative abundance, while root exudates of lettuce (Fig. S1) and garlic (Fig. S2) presented most of the metabolites with peaks

of low relative abundance.

Table 5. Chemical groups and metabolites found in lettuce and garlic root exudates, with and without *B. subtilis* BV09 (Biobaci®), annotated by LC-HRMS/MS, and literature reports about the metabolites bioactivity on *M. incognita*.

Nº	Metabolite name	Peak area					Bioactivity on *M. incognita*
		Lettuce	Lettuce + Bsubt	Garlic	Garlic + Bsubt	Bsubt	
1	4-Guanidinobutyric acid	8.10×10^{7}	1.04×10^{9}	1.31×10^{8}	1.22×10^{9}	3.12×10^{8}	NF
2	N,N-Diethyl-2-aminoethanol	2.18×10^{6}	0	4.10×10^{7}	4.13×10^{7}	0	NF
3	L-Valine	1.31×10^{8}	2.86×10^{8}	4.24×10^{8}	3.27×10^{8}	9.08×10^{8}	Antagonism[a]
4	L-Pipecolic acid	6.39×10^{6}	5.30×10^{7}	0	8.37×10^{7}	2.16×10^{8}	NF
5	Nε-Acetyl-L-lysine	1.27×10^{7}	5.35×10^{7}	0	0	3.23×10^{9}	NF
6	His-Pro	0	1.10×10^{9}	0	4.36×10^{7}	9.37×10^{9}	NF
7	L-Arginine	0	3.99×10^{9}	0	2.12×10^{9}	1.03×10^{10}	Antagonism[b]
8	2-Pyrimidinylacetic acid	1.87×10^{8}	1.17×10^{8}	8.76×10^{7}	5.42×10^{7}	3.41×10^{7}	NF
9	Niacin	1.08×10^{8}	1.33×10^{8}	1.11×10^{8}	7.21×10^{7}	1.76×10^{9}	Attractiveness[c] and Antagonism[d]
10	Pyroglutamic acid	1.44×10^{8}	3.46×10^{8}	6.75×10^{8}	3.02×10^{8}	1.04×10^{9}	NF
11	Adenosine monophosphate	1.18×10^{8}	4.51×10^{8}	0	1.98×10^{6}	2.47×10^{7}	NF
12	Adenosine	2.13×10^{8}	7.15×10^{8}	4.43×10^{8}	4.07×10^{7}	0	NF
13	Cyclo(glycylprolyl)	0	2.29×10^{9}	0	4.06×10^{8}	7.88×10^{9}	NF
14	Pro-Asp	0	2.01×10^{8}	0	1.24×10^{8}	9.20×10^{8}	NF
15	Cyclo-Ala-Pro-diketopiperazine	0	0	0	1.37×10^{8}	2.05×10^{10}	NF
16	Pantothenic acid	0	0	4.88×10^{7}	3.60×10^{7}	1.52×10^{9}	NF
17	Tyr-Pro	0	0	0	9.41×10^{7}	8.79×10^{8}	NF
18	Pro-Val	0	0	0	7.25×10^{7}	6.29×10^{8}	NF
19	PyroGlu-Val	0	7.11×10^{7}	2.36×10^{7}	0	8.28×10^{8}	NF
20	Cyclo(L-prolyl-L-prolyl)	0	6.46×10^{8}	0	3.44×10^{7}	3.49×10^{9}	NF
21	Thr-Leu	0	0	0	8.82×10^{6}	2.42×10^{8}	NF
22	4-Hydroxyquinoline	2.28×10^{7}	6.20×10^{7}	1.93×10^{7}	2.84×10^{7}	4.26×10^{8}	NF
23	Cyclo(prolyltyrosyl)	0	2.38×10^{9}	0	7.31×10^{8}	9.85×10^{9}	NF
24	1,3,8-Trimethyl-3,9-dihydro-1H-purine-2,6-dione	1.34×10^{8}	4.72×10^{7}	2.00×10^{8}	8.09×10^{7}	7.96×10^{7}	NF
25	Cyclo(alanylisoleucyl)	0	5.09×10^{8}	0	1.14×10^{7}	2.44×10^{9}	NF
26	Ile-Pro-Ile	0	8.32×10^{6}	0	0	1.38×10^{9}	NF
27	Cyclo(leucylprolyl)	0	3.06×10^{9}	0	4.29×10^{7}	1.51×10^{10}	NF
28	DL-Prolylphenylalanine	0	8.00×10^{8}	0	4.78×10^{7}	6.00×10^{9}	NF
29	3,5-Dimethylhexanoic acid	1.44×10^{8}	3.94×10^{7}	4.94×10^{7}	4.50×10^{7}	7.84×10^{7}	NF
30	Valylphenylalanine	0	2.97×10^{7}	0	0	1.98×10^{8}	NF
31	(2E)-4-Hydroxynon-2-enoic acid	1.11×10^{8}	7.46×10^{7}	1.96×10^{8}	3.72×10^{7}	4.60×10^{7}	NF
32	1-(2-Hydroxy-4,5-dimethylphenyl)ethanone	2.99×10^{8}	9.38×10^{7}	0	2.28×10^{7}	0	NF
33	Tuberonic acid glucoside	0	0	1.75×10^{8}	1.95×10^{6}	0	NF
34	3,4-Dimethylbenzaldehyde	2.49×10^{8}	9.78×10^{7}	4.99×10^{8}	3.18×10^{8}	1.15×10^{8}	NF

Rt – Retention time; Bsubt – *B. subtilis* BV09 (Biobaci®) application; NF – not found. [a] Osman and Viglierchio (1981), [b] Al-Sayed and Thomason (1988), [c] Kuang et al. (2020) and [d] Montasser (1990).

Among the differential metabolites, some were observed in lettuce and garlic root exudates only when *B. subtilis* BV09 was applied, while others were not found in garlic or lettuce root exudates, even with the application of *B. subtilis* BV09. On the other hand, some metabolites were observed in lettuce and garlic root exudates with and without the application of *B. subtilis* BV09, but presented larger or smaller peak area when *B. subtilis* BV09 was applied in lettuce and garlic roots (Table 5).

Amino acids and peptides was the majority chemical group in all treatments, being more expressive in the treatments with the presence of *B. subtilis* BV09 (T2, T4 and T5). Chemical groups from primary metabolism were found in all treatments. For secondary metabolites, phenolic compounds were not found in all treatments, being found in lettuce root exudates with and without *B. subtilis* BV09 (T2 and T1, respectively), while in garlic root exudates phenolic compounds was found only in the presence of *B. subtilis* BV09 (T4). The phytohormone tuberonic acid glucoside was only found in garlic root exudates, showing smaller peak area when *B. subtilis* BV09 was applied (Table 5 and Fig. 3).

The *M. incognita* chemotactic response to the metabolites found in *B. subtilis* BV09 suspension and in lettuce and garlic root exudates was rarely studied, being found only one research reporting attractiveness of niacin. Metabolites antagonism to *M. incognita* was also rarely found in the literature, with only three researches reporting antagonism of L-valine, L-arginine and niacin (Table 5).

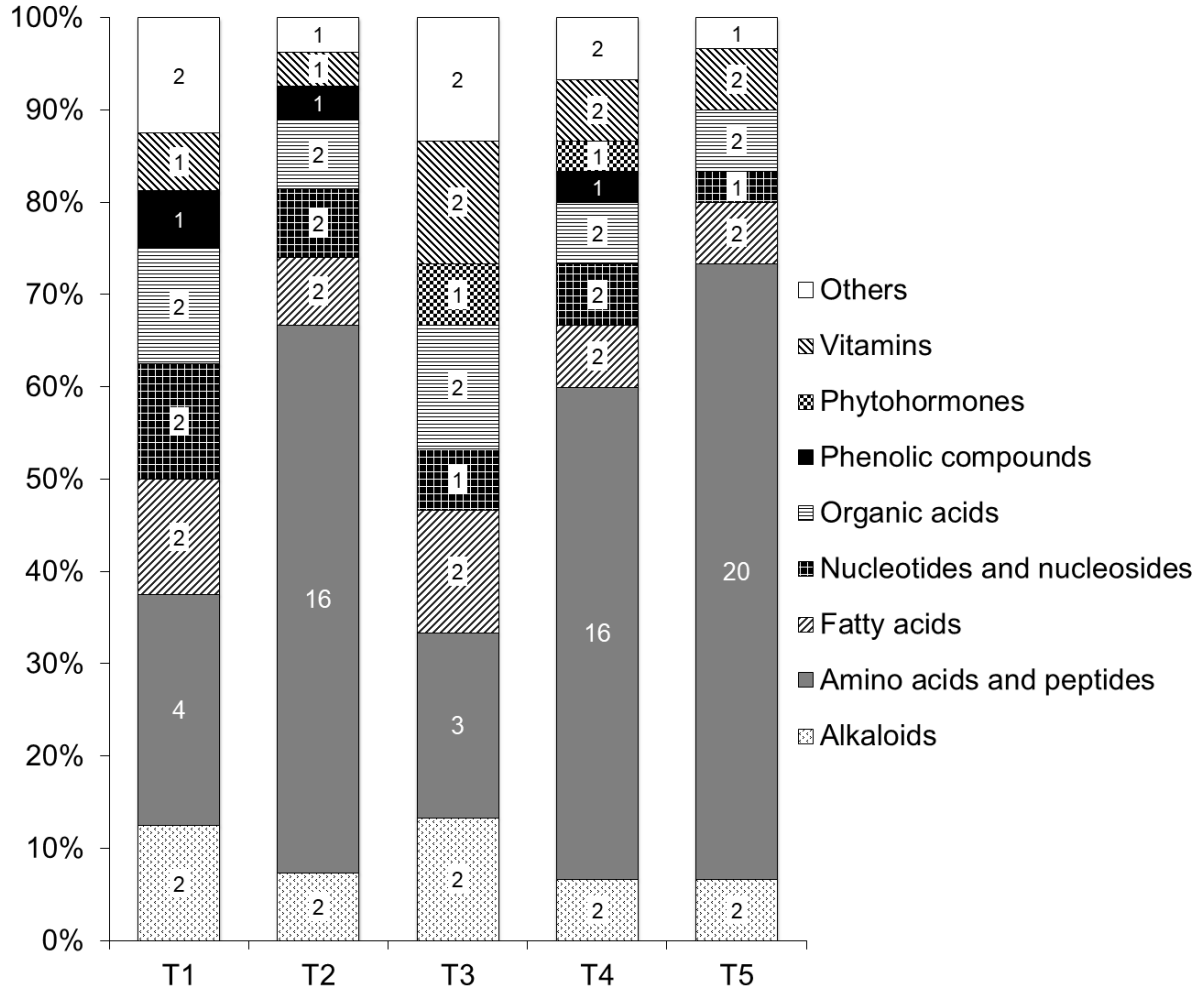

Fig. 3. Percentage and number of metabolites of each chemical group in the different treatments. T1 = Root exudates of lettuce plants; T2 = Root exudates of lettuce plants with the application of *B. subtilis* BV09 (Biobaci®); T3 = Root exudates of garlic plants; T4 = Root exudates of garlic plants with the application of *B. subtilis* BV09; T5 = Suspension of *B. subtilis* BV09.

4. Discussion

Plants use their root exudates to interact with other organisms in the soil. Plant-parasitic nematodes (PPN) move on the ground to find their hosts through chemotactic behavior responding to plant roots signals (Haichar et al., 2014; Wang et al., 2021). However, some non-host plants can release in the soil metabolites to avoid root-knot nematode (RKN) juveniles find their roots (Torto et al., 2018), as observed for garlic root exudates in this work.

The differentiated response of *M. incognita* second-stage juveniles (J2) to lettuce and garlic root exudates may be related to the specificity of each plant species root exudates

295 composition (Wang et al., 2021), being observed some specific metabolites of lettuce or garlic
296 plants when performing the metabolomics analysis. In the same way, lettuce and garlic had
297 changes in root exudates composition when *B. subtilis* BV09 (Biobaci®) was applied in their
298 roots (Table 5), which may explain the different response of *M. incognita* J2 observed for the
299 root exudates collected with the presence or absence of *B. subtilis* BV09 (Fig. 2). In
300 belowground plant-microbe interactions, plants release root exudates that recruit beneficial
301 microorganisms, such as *B. subtilis* BV09, and favor their establishment and biofilm
302 formation on plant roots, helping in controlling diseases and promoting plant growth (Haichar
303 et al., 2014; Martins et al., 2018; Sun et al., 2021; Vives-Peris et al., 2020).

304 Lettuce is a host of *M. incognita* (Sgorlon et al., 2018), which in part may be related to
305 its root exudates' attractiveness for the *M. incognita* J2. The attractiveness of *M. incognita* J2
306 to lettuce roots has already been observed by Wang et al. (2021). Interestingly, when *B.*
307 *subtilis* BV09 was applied to lettuce roots its root exudates showed repellency for *M.*
308 *incognita* J2 (Fig. 2). Therefore, the changes on lettuce root exudates caused by *B. subtilis*
309 application can lead to nematode disorientation (Araújo and Marchesi, 2009). The repellence
310 response of *M. incognita* J2 to *B. subtilis* BV09 suspension may also explain the repellency of
311 lettuce root exudates when *B. subtilis* BV09 was applied, being found reports in the literature
312 of the repellence of *Bacillus* spp. against the *M. incognita* J2s (Cao et al., 2019; Hu et al.,
313 2017). Thus, *Bacillus* spp. can protect plant roots by colonizing them through biofilm
314 formation, in addition to releasing metabolites with nematicidal and disorienting action
315 (Júnior et al., 2022).

316 On the other hand, garlic root exudates were repellent for *M. incognita* J2, which may
317 be related to garlic being an antagonistic plant to *M. incognita* (El-Saedy et al., 2014), with
318 repellency of the *M. incognita* J2 already observed to other non-host plants (Mwamba et al.,
319 2021). However, when *B. subtilis* BV09 was applied to garlic roots its exudates showed

attractiveness for *M. incognita* J2 (Fig. 2). Rhizosphere bacteria have the ability to colonize plant roots and can cause degradation of specific root exudates metabolites (Oostendorp and Sikora, 1990), which may have occurred when *B. subtilis* BV09 was applied to garlic roots, degrading the metabolites responsible for its repellent action to *M. incognita* J2.

The chemical group that presented the highest diversity of metabolites in lettuce and garlic root exudates was amino acids and peptides, mainly when *B. subtilis* BV09 was applied in lettuce and garlic roots. The amino acids give rise to the precursors of many defense metabolites (Waqas et al., 2015), and some peptides produced by *B. subtilis* have antimicrobial properties (Gowda et al., 2022). Thus, the inoculation of endophytic bacteria, such as *Bacillus* spp., may increase the content of amino acids and peptides in plant tissues, playing important roles in plant immunity against pathogens (Gowda et al., 2022; Pandey et al., 2019; Waqas et al., 2015).

Some amino acids found in the root exudates of lettuce and garlic inoculated with *B. subtilis* BV09 (Table 5) can act directly against of RKN, such as DL-Valine and DL-Arginine, with a report of their activity reducing *M. javanica* eggs hatching and J2 viability *in vitro*, as well as they can act inducing plant tolerance, being found a report of these amino acids reducing galling, egg mass formation and J2 population in the soil through their foliar application on tomato plants (Selim et al., 2019).

Plant growth-promoting rhizobacteria (PGPR) can enhance plant resistance to oxidative stress through its ability to produce antioxidant metabolites, such as the water-soluble vitamins niacin and pantothenic acid (Hayat et al., 2012). In this work, niacin and pantothenic acid were observed in the suspension of *B. subtilis* BV09, as well as in the root exudates (Table 5). The nicotinic acid (niacin) has the ability to reduce the number of galls and *M. incognita* reproduction on tomato plants (Montasser, 1990) and can be attractive to *M. incognita* J2 (Kuang et al., 2020).

Phenolic compounds are secondary metabolites important in plant defense response, which act avoiding pathogenic infections and protecting plant tissues from toxic effect of reactive oxygen species (ROS), being rapidly accumulated by plants in response to environmental stress (Kumar et al., 2020). The phenolic compound 1-(2-Hydroxy-4,5-dimethylphenyl)ethanone was not observed in garlic root exudates, being found only when *B. subtilis* BV09 was applied in garlic roots (Table 5). The application of biological control agents, such as *B. subtilis*, may increase garlic bioactive compound production and improve antioxidant activity, in addition to helping control plant pathogens (Cavalcanti et al., 2020).

As the root exudates composition, metabolite concentrations also influence the J2 response, being reported by several researchers some concentration-dependent responses (Kirwa et al., 2018; Shivakumara et al., 2018; Zhai et al., 2018). For example, Kirwa et al. (2018) observed attractiveness at low quercetin concentrations, while at high quercetin concentrations there was repellency of *M. incognita* J2.

For the nucleotides and nucleosides, an increase in adenosine and adenosine monophosphate was observed in lettuce root exudates when *B. subtilis* BV09 was applied in lettuce roots 24h before of root exudates collection (Table 5). Misaghi et al. (1975) showed that a sharp increase in the levels of total adenylates 24 h after infection of *M. incognita* in cotton roots might be attributed to a defensive reaction. Therefore, the application of *B. subtilis* BV09 may be inducing plant defense response.

Pipecolic acid play important role in defense amplification, showing positive regulation of salicylic acid biosynthesis and inducing defense priming, improving the defensive capacity of plants (Návarová et al., 2012). Pyroglutamic acid was found in tomato plants after infection by *M. incognita* (Eloh et al., 2016), and 4-Guanidinobutyric acid was found in *M. incognita* resistance peach plants in higher amounts than in susceptible peach plants (Liu et al., 2020), thus these metabolites may be related to plant defense response.

Therefore, the increase of L-Pipecolic acid, pyroglutamic acid and 4-Guanidinobutyric acid observed in lettuce root exudates in the presence of *B. subtilis* BV09, as well as the increase of 4-Guanidinobutyric acid and the production of L-Pipecolic acid in garlic root exudates in the presence of *B. subtilis* BV09 (Table 5) may be involved in lettuce and garlic defense response.

Tuberonic acid glucoside, also known as 12-hydroxyjasmonic acid, is involved in plant defense responses (Aprile et al., 2022). Its formation depends on jasmonic acid (JA) synthesis, and its accumulation occur when JA is hydroxylated, forming 12-hydroxyjasmonic acid and down-regulating wound-response (Miersch et al., 2008). Therefore, the lower amount of tuberonic acid glucoside when *B. subtilis* BV09 was applied in garlic roots (Table 5) may indicate that there was less formation of JA, or it may indicate that there was less hydroxylation of JA, consequently maintaining active the plant defense system signaling some stress in the presence of *B. subtilis* BV09.

L-Arginine can reduce the percentage of root galls and *M. javanica* J2 in soil, improve plant growth response of tomato plants and suppress *M. javanica* infection through the stimulation of tomato tolerance (Shaimaa et al., 2021). The increase in L-Valine concentration led to a reduction in the number of egg masses of *M. incognita* in sunflower plants (Osman and Viglierchio, 1981). L-Arginine was found in *B. subtilis* BV09 suspension, being found in lettuce and garlic root exudates when *B. subtilis* BV09 was applied, while L-Valine was found in all samples, being metabolites of great interest in the management of *Meloigodyne* spp. (Table 5).

Some metabolites can act directly on the nematode, such as L-Arginine, which act inhibiting the eggs hatching and causing mortality of *M. incognita* J2 (Al-Sayed and Thomason, 1988). The fatty acids palmitic acid and linoleic acid released from castor (*Ricinus communis* L.) were observed by Dong et al. (2018) presenting nematicidal action for *M.*

incognita and negative chemotaxis repelling the J2 in a concentration-dependent response. While for the fatty acids observed in lettuce and garlic root exudates with and without *B. subtilis* BV09, 3,5-Dimethylhexanoic acid and (2E)-4-Hydroxynon-2-enoic acid, were not found reports of their biological activities (Table 5).

5. Conclusion

Metabolomics analysis showed great changes in metabolites in lettuce and garlic root exudates caused by *Bacillus subtilis* BV09 inoculation. The application of *B. subtilis* BV09 in lettuce and garlic roots affected *Meloidogyne incognita* chemotactic response to their root exudates since modified its chemical composition. In the presence of *B. subtilis* BV09, lettuce root exudates stopped being attractive and became repellent to *M. incognita*, while garlic root exudates stopped being repellent and became attractive to *M. incognita*.

A knowledge gap was found about the biological activities of some metabolites present in the root exudates of lettuce and garlic, as well as in *B. subtilis* BV09 suspension. Then, further studies are needed to resolve the doubt about the chemotactic response of *M. incognita* to these metabolites and if they have nematicidal action.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. The authors thank the CAPES, FAPEMIG and CNPq institutions for granting scholarships and for supporting research in our institution. Thanks to Grupo Vittia for providing the Biobaci® and technical support. Thanks to the Laboratory of Nematology of the Plant Pathology Department (DFP) and the Plant Tissue Culture Laboratory of the Department of Agriculture (DAG) of the Federal University of Lavras (Universidade Federal de Lavras - UFLA) for the structure provided for the

experiments and for the support of the technicians and teachers. Thanks to Prof. Dr. Rafael Garrett da Costa from UFRJ for helping with the metabolomics analysis. Thanks to Lílian Ferreira de Sousa, Regiane Alves Belizario, Kamilly Maria Fernandes Fonseca, Brenda Miriam Silva and Daiane dos Santos Soares for assisting in the laboratory activities.

References

Al-Sayed, A.A., Thomason, I.J., 1988. Meloidogyne incognita and Tomato Response to Thiamine, Ascorbic Acid, L-arginine, and L-glutamic Acid. J. Nematol. 20, 451–456.

Ali, J.G., Alborn, H.T., Stelinski, L.L., 2011. Constitutive and induced subterranean plant volatiles attract both entomopathogenic and plant parasitic nematodes. J. Ecol. 99, 26–35. https://doi.org/10.1111/j.1365-2745.2010.01758.x

Ansari, R.A., Rizvi, R., Sumbul, A., Mahmood, I., 2020. Plant-Growth-Promoting Rhizobacteria (PGPR)-Based Sustainable Management of Phytoparasitic Nematodes: Current Understandings and Future Challenges, in: Ansari, R.A., Rizvi, R., Mahmood, I. (Eds.), Management of Phytonematodes : Recent Advances and Future Challenges. Springer, pp. 51–71.

Aprile, A.M., Coppola, M., Turrà, D., Vitale, S., Cascone, P., Diretto, G., Fiore, A., Castaldi, V., Romanelli, A., Avitabile, C., Guerrieri, E., Lois, S., Rao, R., Maria, A., Coppola, M., Turrà, D., Vitale, S., Avitabile, C., Guerrieri, E., Woo, S.L., Rao, R., 2022. Combination of the Systemin peptide with the beneficial fungus Trichoderma afroharzianum T22 improves plant defense responses against pests and diseases. J. Plant Interact. 17, 569–579. https://doi.org/10.1080/17429145.2022.2072528

Araújo, F.F. de, Marchesi, G.V.P., 2009. Uso de Bacillus subtilis no controle da meloidoginose e na promoção do crescimento do tomateiro. Ciência Rural 39, 1558–1561. https://doi.org/10.1590/s0103-84782009000500039

Basyony, A.G., Abo-Zaid, G.A., 2018. Biocontrol of the root-knot nematode, Meloidogyne incognita, using an eco-friendly formulation from Bacillus subtilis, lab. and greenhouse studies. Egypt. J. Biol. Pest Control 28, 87. https://doi.org/10.1186/s41938-018-0094-4

Cao, H., Jiao, Y., Yin, N., Li, Y., Ling, J., Mao, Z., Yang, Y., Xie, B., 2019. Analysis of the activity and biological control efficacy of the Bacillus subtilis strain Bs-1 against Meloidogyne incognita. Crop Prot. 122, 125–135. https://doi.org/10.1016/j.cropro.2019.04.021

Cavalcanti, V.P., Aazza, S., Bertolucci, S.K.V., Pereira, M.M.A., Cavalcanti, P.P., Buttrós, V.H.T., e Silva, A.M. de O., Pasqual, M., Dória, J., 2020. Plant, pathogen and biocontrol agent interaction effects on bioactive compounds and antioxidant activity in garlic. Physiol. Mol. Plant Pathol. 112, 101550. https://doi.org/10.1016/j.pmpp.2020.101550

Čepulyte, R., Danquah, W.B., Bruening, G., Williamson, V.M., 2018. Potent Attractant for Root-Knot Nematodes in Exudates from Seedling Root Tips of Two Host Species. Sci. Rep. 8. https://doi.org/10.1038/s41598-018-29165-4

Charchar, J.M., Moita, A.W., 2005. Metodologia para seleção de hortaliças com resistência a nematóides: Alface/Meloidogyne spp. Embrapa, Brasília.

Chitwood, D.J., 2002. PHYTOCHEMICAL BASED STRATEGIES FOR NEMATODE CONTROL. Annu. Rev. Phytopathol. 40, 221–249. https://doi.org/10.1146/annurev.phyto.40.032602.130045

D'Addabbo, T., Laquale, S., Lovelli, S., Candido, V., Avato, P., 2014. Biocide plants as a

sustainable tool for the control of pests and pathogens in vegetable cropping systems. Ital. J. Agron. 9, 137–145. https://doi.org/10.4081/ija.2014.616

Delory, B.M., Delaplace, P., Fauconnier, M.-L., du Jardin, P., 2016. Root-emitted volatile organic compounds: can they mediate belowground plant-plant interactions? Plant Soil 402, 1–26. https://doi.org/10.1007/s11104-016-2823-3

Dong, L., Li, X., Huang, C., Lu, Q., Li, B., Yao, Y., Liu, T., Zuo, Y., 2018. Reduced Meloidogyne incognita infection of tomato in the presence of castor and the involvement of fatty acids. Sci. Hortic. (Amsterdam). 237, 169–175. https://doi.org/10.1016/j.scienta.2018.03.066

Eder, R., Consoli, E., Krauss, J., Dahlin, P., 2021. Polysulfides Applied as Formulated Garlic Extract to Protect Tomato Plants against the Root-Knot Nematode Meloidogyne incognita. Plants 10, 394. https://doi.org/10.3390/plants10020394

El-Saedy, M.A.M., Mokbel, A.A., Hammad, S.E., 2014. Efficacy of plant oils and garlic cultivation on controlling Meloidogyne incognita infected tomato plants. Pakistan J. Nematol. 32, 39–50.

Eloh, K., Sasanelli, N., Maxia, A., Caboni, P., 2016. Untargeted Metabolomics of Tomato Plants after Root-Knot Nematode Infestation. J. Agric. Food Chem. Nematode 64, 5963–5968. https://doi.org/10.1021/acs.jafc.6b02181

Gowda, M.T., Meena, B.R., Krishnan, N., Manjunath, M., Sellaperumal, C., Rai, A.B., Singh, A., Manimurugan, C., Patil, J., Pandey, K.K., Singh, J., 2022. Antimicrobial peptides producing native Bacillus spp. for the management of root-knot nematode Meloidogyne incognita infecting okra (Abelmoschus esculentus L. Moench). Biol. Control 171, 104951. https://doi.org/10.1016/j.biocontrol.2022.104951

Haichar, F. el Z., Santaella, C., Heulin, T., Achouak, W., 2014. Root exudates mediated interactions belowground. Soil Biol. Biochem. 77, 69–80. https://doi.org/10.1016/j.soilbio.2014.06.017

Hayat, R., Ahmed, I., Sheirdil, R.A., 2012. An Overview of Plant Growth Promoting Rhizobacteria (PGPR) for Sustainable Agriculture, in: Crop Production for Agricultural Improvement. pp. 557–579. https://doi.org/10.1007/978-94-007-4116-4

Hu, H.-J., Chen, Y.-L., Wang, Y.-F., Tang, Y.-Y., Chen, S.-L., Yan, S.-Z., 2017. Endophytic Bacillus cereus Effectively Controls Meloidogyne incognita on Tomato Plants Through Rapid Rhizosphere Occupation and Repellent Action. Plant Dis. 101, 448–455. https://doi.org/10.1094/PDIS-06-16-0871-RE

Hussey, R.S., Barker, K.R., 1973. A comparison of methods for colecting inocula of meloidogyne spp including a new technique. Plant Dis. Report. 57, 1025–1028.

Jardim, I.N., Oliveira, D.F., Campos, V.P., Silva, G.H., Souza, P.E., 2020. Garlic essential oil reduces the population of Meloidogyne incognita in tomato plants. Eur. J. Plant Pathol. 157, 197–209. https://doi.org/10.1007/s10658-020-02000-1

Júnior, O.V. de C., Sá, A.V. de, Peixoto, A.R., Paz, C.D. da, Castro, J.M. da C. e, Gava, C.A.T., 2022. Local Bacillus species as potential biocontrol agents for Meloidogyne enterolobii in melon (Cucumis melo L.). Biocontrol Sci. Technol. 32, 314–328. https://doi.org/10.1080/09583157.2021.1999394

Kirwa, H.K., Murungi, L.K., Beck, J.J., Torto, B., 2018. Elicitation of Differential Responses in the Root-Knot Nematode Meloidogyne incognita to Tomato Root Exudate Cytokinin, Flavonoids, and Alkaloids. J. Agric. Food Chem. 66, 11291–11300. https://doi.org/10.1021/acs.jafc.8b05101

Kouamé, A.P., 2021. Effectiveness of Garlic and Onion Aqueous Extracts on Tomato Root-Knot Nematodes (Meloidogyne sp.) in the Autonomous District of Yamoussoukro in Central Côte d'Ivoire. Indian J. Pure Appl. Biosci. 9, 24–35. https://doi.org/10.18782/2582-2845.8532

Kuang, M., Liu, T., Wu, Hai-bin, Lan, H., Wen, Y., Wu, Hai-bo, Li, X., 2020. Constituents Leached by Tomato Seeds Regulate the Behavior of Root-Knot Nematodes and Their Antifungal Effects against Seed-Borne Fungi. J. Agric. Food Chem. 68, 9061–9069. https://doi.org/10.1021/acs.jafc.0c01797

Kumar, S., Abedin, M.M., Singh, A.K., Das, S., 2020. Role of Phenolic Compounds in Plant-Defensive Mechanisms, in: Lone, R., Shuab, R., Kamili, A.N. (Eds.), Phenolics in Sustainable Agriculture. pp. 517–532.

Liu, K., Wang, L., Gengrui, Z., Weichao, F., Yong, L., Ke, C., 2020. Identification of the metabolites involved in resistance to Meloidogyne incognita in the 'Honggengansutao 1' peach. J. Fruit Sci. 37, 254–263. https://doi.org/10.13925/j.cnki.gsxb.2019020190488

Liu, W., Jones, A.L., Gosse, H.N., Lawrence, K.S., Park, S.-W., 2019. Validation of the Chemotaxis of Plant Parasitic Nematodes Toward Host Root Exudates. J. Nematol. 51, 1–10. https://doi.org/10.21307/jofnem-2019-063

Martins, S.J., Medeiros, F.H.V., Lakshmanan, V., Bais, H.P., 2018. Impact of seed exudates on growth and biofilm formation of Bacillus amyloliquefaciens ALB629 in common bean. Front. Microbiol. 8, 1–9. https://doi.org/10.3389/fmicb.2017.02631

Miersch, O., Neumerkel, J., Dippe, M., Stenzel, I., Wasternack, C., 2008. Hydroxylated jasmonates are commonly occurring metabolites of jasmonic acid and contribute to a partial switch-off in jasmonate signaling. New Phytol. 177, 114–127. https://doi.org/10.1111/j.1469-8137.2007.02252.x

Migunova, V.D., Sasanelli, N., 2021. Bacteria as biocontrol tool against phytoparasitic nematodes. Plants 10, 1–15. https://doi.org/10.3390/plants10020389

Misaghi, I., McClure, M.A., Kruk, T.H., 1975. Concentration of adenylates and energy charge values in cotton roots infected with Meloidogyne incognita. Physiol. Plant Pathol. 6, 85–91.

Montasser, S.A., 1990. Efficacy of certain vitamins in controlling the root-knot nematode, Meloidogyne incognita on tomato. Pakistan J. Nematol. 8, 101–105.

Moosavi, M.R., 2020. Efficacy of Microbial Biocontrol Agents in Integration with Other Managing Methods against Phytoparasitic Nematodes, in: Ansari, R.A., Rizvi, R., Mahmood, I. (Eds.), Management of Phytonematodes : Recent Advances and Future Challenges. Springer, pp. 229–258.

Mwamba, S., Kihika-opanda, R., Murungi, L.K., Losenge, T., Beck, J.J., Torto, B., 2021. Identification of Repellents from Four Non-Host Asteraceae Plants for the Root Knot Nematode, Meloidogyne incognita. J. Agric. Food Chem. 69, 15145–15156. https://doi.org/10.1021/acs.jafc.1c06500

Návarová, H., Bernsdorff, F., Döring, A.-C., Zeier, J., 2012. Pipecolic Acid, an Endogenous Mediator of Defense Ampli fi cation and Priming, Is a Critical Regulator of Inducible Plant Immunity. Plant Cell 24, 5123–5141. https://doi.org/10.1105/tpc.112.103564

Oostendorp, M., Sikora, R.A., 1990. In-vitro interrelationships between rhizosphere bacteria and Heterodera schachtii. Rev. Nématologie 13, 269–274.

Osman, A.A., Viglierchio, D.R., 1981. Foliar Spray Effects of Selected Amino Acids on Sunflower Infected with Meloidogyne incognita. J. o I Nematol. 13, 3–5.

Pandey, P.K., Samanta, R., Narain, R., Yadav, S., 2019. Inside the plant : addressing bacterial endophytes in biotic stress alleviation. Arch. Microbiol. 201, 415–429. https://doi.org/10.1007/s00203-019-01642-y

Pinheiro, J.B., Pereira, R.B., Suinaga, F.A., 2014. Manejo de nematoides na cultura do tomate, 1st ed. Embrapa Hortaliças, Brasília.

R Core Team, 2019. R: A language and environment for statistical computing [WWW Document]. URL https://www.r-project.org/

Rasmann, S., Ali, J.G., Helder, J., van der Putten, W.H., 2012. Ecology and Evolution of Soil

Nematode Chemotaxis. J. Chem. Ecol. 38, 615–628. https://doi.org/10.1007/s10886-012-0118-6

Rovira, A.D., 1969. Plant root exudates. Bot. Rev. 35, 35–57. https://doi.org/10.1007/BF02859887

Selim, M.E., Galal, N.M., Mahdy, M.E., 2019. The potential role of some amino acids with different concentrations , application types and times to manage Meloidogyne javanica on tomato plants. Egypt. J. Crop Prot. 14, 11–24.

Sgorlon, L.F.F., Silva, E.H.C., Soares, R.S., Borges, H.O., Diniz, G.M.M., Braz, L.T., Soares, P.L.M., 2018. Host status of crispy-leaf lettuce cultivars to root-knot nematodes. Biosci. J. 34, 1319–1325. https://doi.org/10.14393/BJ-v34n5a2018-39387

Shaimaa, N., El-zawahry, A.M.I., Aref, H.M. El, Hanaa, S., 2021. Induction of Systemic Resistance in Tomato by some Abiotic Compounds Against Meloidogyne javanica. Assiut J. Agric. Sci. 52, 74–89. https://doi.org/10.21608/ajas.2021.171570

Shivakumara, T.N., Dutta, T.K., Rao, U., 2018. A novel in vitro chemotaxis bioassay to assess the response of Meloidogyne incognita towards various test compounds. J. Nematol. 50, 487–494. https://doi.org/10.21307/jofnem-2018-047

Siddique, S., Coomer, A., Baum, T., Williamson, V.M., 2022. Recognition and Response in Plant–Nematode Interactions. Annu. Rev. ofPhytopathology 60, 1–20.

Sikder, M., Vestergård, M., 2020. Impacts of Root Metabolites on Soil Nematodes. Front. Plant Sci. 10, 1–18. https://doi.org/10.3389/fpls.2019.01792

Sikora, R.A., Fernandez, E., 2005. Nematode parasites of vegetables, in: Luc, M., Sikora, R.A., Bridge, J. (Eds.), Plant-Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International, Wallingford, UK, pp. 319–391.

Sun, H., Jiang, S., Jiang, C., Wu, C., Gao, M., Wang, Q., 2021. A review of root exudates and rhizosphere microbiome for crop production. Environ. Sci. Pollut. Res. 28, 54497–54510. https://doi.org/10.1007/s11356-021-15838-7

Torto, B., Kirwa, H., Kihika, R., Murungi, L.K., 2018. Strategies for the Manipulation of Root Knot Nematode Behavior with Natural Products in Small Scale Farming Systems. ACS Symp. Ser. 1294, 114–126. https://doi.org/10.1021/bk-2018-1294.ch009

Vives-Peris, V., Ollas, C. de, Gómez-Cadenas, A., Pérez-Clemente, R.M., 2020. Root exudates: from plant to rhizosphere and beyond. Plant Cell Rep. 39, 3–17. https://doi.org/10.1007/s00299-019-02447-5

Wang, J., Ding, Z., Bian, J., Bo, T., Liu, Y., 2021. Chemotaxis response of Meloidogyne incognita to volatiles and organic acids from root exudates. Rhizosphere 17, 100320. https://doi.org/10.1016/j.rhisph.2021.100320

Wang, P., Sun, Y., Yang, L., Hu, Y., Li, J., Wang, J., 2019. Chemotactic responses of the root-knot nematode Meloidogyne incognita to Streptomyces plicatus. FEMS Microbiol. Lett. 366, 1–7. https://doi.org/10.1093/femsle/fnz234

Waqas, M., Khan, A.L., Hamayun, M., Shahzad, R., Kim, Y., Choi, K., Lee, I., 2015. Endophytic infection alleviates biotic stress in sunflower through regulation of defence hormones , antioxidants and functional amino acids. Eur. J. Plant Pathol. 141, 803–824. https://doi.org/10.1007/s10658-014-0581-8

Zhai, Y., Shao, Z., Cai, M., Zheng, L., Li, G., Huang, D., Cheng, W., Thomashow, L.S., Weller, D.M., Yu, Z., Zhang, J., 2018. Multiple Modes of Nematode Control by Volatiles of Pseudomonas putida 1A00316 from Antarctic Soil against Meloidogyne incognita. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.00253

Supplementary material

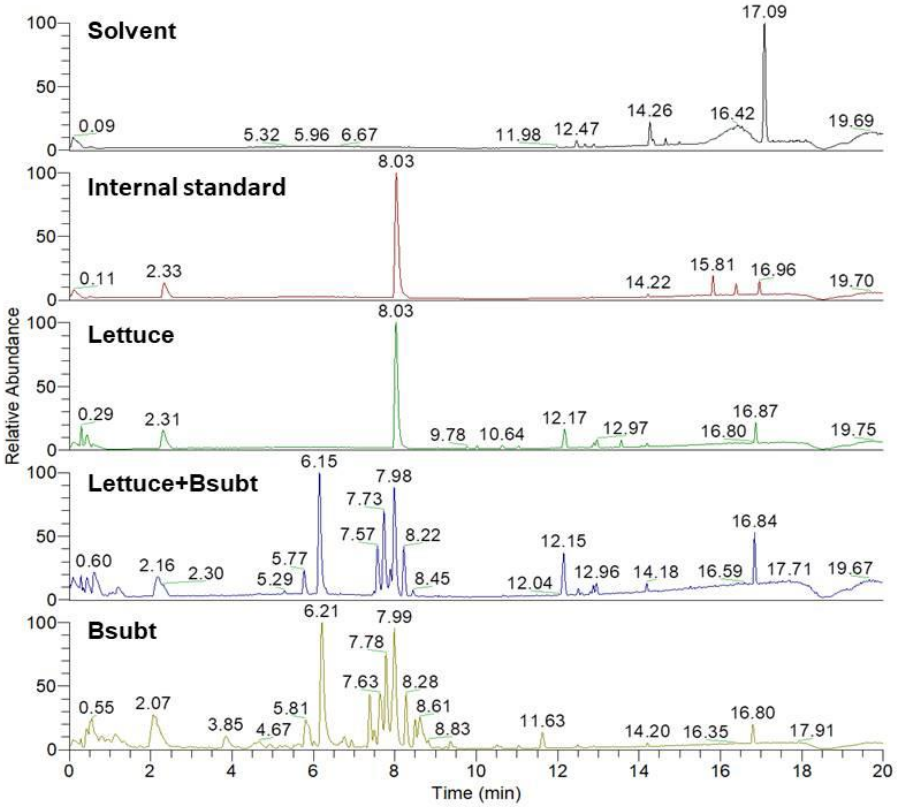

Fig. S1. Typical LC-HRMS chromatograms of lettuce root exudates with and without the application of *B. subtilis* BV09. Solvent – H_2O:MeOH; Internal standard – p-fluoro-DL-phenylalanine; Lettuce – lettuce root exudates; Lettuce+Bsubt – lettuce root exudates with the application of *B. subtilis* BV09; Bsubt – *B. subtilis* BV09 suspension.

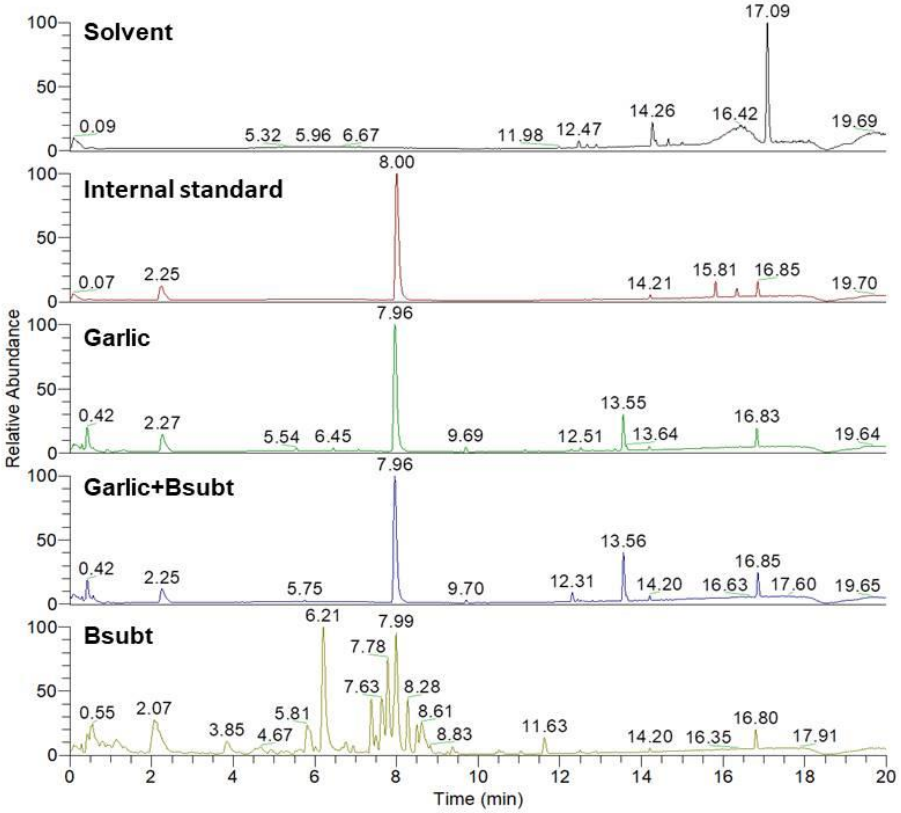

Fig. S2. Typical LC-HRMS chromatograms of garlic root exudates with and without the application of *B. subtilis* BV09. Solvent – H_2O:MeOH; Internal standard – p-fluoro-DL-phenylalanine; Garlic – garlic root exudates; Garlic+Bsubt – garlic root exudates with the application of *B. subtilis* BV09; Bsubt – *B. subtilis* BV09 suspension.

Table S1. List of putatively identified compounds by LC-HRMS/MS in lettuce and garlic root exudates, with and without *B. subtilis* BV09 application.

Nº	Metabolite name	Rt (min)	Experimental *m/z*	Theoretical *m/z*	Mass error (ppm)	Formula
1	4-Guanidinobutyric acid	0.47	146.0923	146.0924	-0.82	$C_5H_{11}N_3O_2$
2	N,N-Diethyl-2-aminoethanol	0.47	118.1231	118.1226	4.49	$C_6H_{15}NO$
3	L-Valine	0.49	118.0867	118.0863	3.56	$C_5H_{11}NO_2$
4	L-Pipecolic acid or L-Lysine	0.50	130.0864	130.0863	0.77	$C_6H_{11}NO_2$
5	Nε-Acetyl-L-lysine	0.52	189.1238	189.1234	2.27	$C_8H_{16}N_2O_3$
6	His-Pro	0.54	235.1191	235.1190	0.21	$C_{11}H_{16}N_4O_3$
7	L-Arginine	0.58	158.0924	158.0924	0.06	$C_6H_{14}N_4O_2$
8	2-Pyrimidinylacetic acid	0.60	121.0398	121.0396	1.65	$C_6H_6N_2O_2$
9	Niacin	0.62	124.0397	124.0393	2.98	$C_6H_5NO_2$
10	Pyroglutamic acid	0.67	130.0501	130.0499	1.77	$C_5H_7NO_3$
11	Adenosine monophosphate	0.71	348.0706	348.0704	0.63	$C_{10}H_{14}N_5O_7P$
12	Adenosine	1.04	268.1045	268.1040	1.94	$C_{10}H_{13}N_5O_4$
13	Cyclo(glycylprolyl)	1.16	155.0817	155.0815	1.35	$C_7H_{10}N_2O_2$
14	Pro-Asp	1.29	213.0875	213.0870	2.49	$C_9H_{14}N_2O_5$
15	3-Methylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione	2.07	169.0969	169.0972	-1.54	$C_8H_{12}N_2O_2$
16	Pantothenic acid	2.99	220.1183	220.1180	1.32	$C_9H_{17}NO_5$
17	Tyr-Pro	3.35	279.1348	279.1339	3.30	$C_{14}H_{18}N_2O_4$
18	Pro-Val	3.42	215.1391	215.1390	0.60	$C_{10}H_{18}N_2O_3$
19	PyroGlu-Val	4.56	229.1190	229.1183	2.92	$C_{10}H_{16}N_2O_4$
20	Cyclo(L-prolyl-L-prolyl)	4.68	195.1131	195.1128	1.49	$C_{10}H_{14}N_2O_2$
21	Thr-Leu	5.07	233.1502	233.1496	2.62	$C_{10}H_{20}N_2O_4$
22	4-Hydroxyquinoline	5.14	146.0604	146.0600	2.67	C_9H_7NO
23	Cyclo(prolyltyrosyl)	5.78	261.1246	261.1234	4.67	$C_{14}H_{16}N_2O_3$
24	1,3,8-Trimethyl-3,9-dihydro-1H-purine-2,6-dione	6.71	195.0876	195.0877	-0.72	$C_8H_{10}N_4O_2$
25	Cyclo(alanylisoleucyl)	6.89	185.1289	185.1285	2.43	$C_9H_{16}N_2O_2$
26	Ile-Pro-Ile	7.68	342.2388	342.2387	0.35	$C_{17}H_{31}N_3O_4$
27	Cyclo(leucylprolyl)	7.91	211.1443	211.1441	0.95	$C_{11}H_{18}N_2O_2$
28	DL-Prolylphenylalanine	8.47	245.1292	245.1285	2.90	$C_{14}H_{18}N_2O_3$
29	3,5-Dimethylhexanoic acid	8.61	109.1017	109.1012	4.40	$C_8H_{16}O_2$
30	Valylphenylalanine	9.76	247.1452	247.1441	4.29	$C_{14}H20N_2O_3$
31	(2E)-4-Hydroxynon-2-enoic acid	10.49	155.1068	155.1067	0.45	$C_9H_{16}O_3$
32	1-(2-Hydroxy-4,5-dimethylphenyl)ethanone	10.62	165.0909	165.0910	-0.91	$C_{10}H_{12}O_2$
33	Tuberonic acid glucoside	11.24	389.1793	389.1806	-3.44	$C_{18}H_{28}O_9$
34	3,4-Dimethylbenzaldehyde	12.52	135.0806	135.0804	1.48	$C_9H_{10}O$

Smart Agricultural Technology 3 (2023) 100100

Contents lists available at ScienceDirect

Smart Agricultural Technology

journal homepage: www.journals.elsevier.com/smart-agricultural-technology

Use of RGB images from unmanned aerial vehicle to estimate lettuce growth in root-knot nematode infested soil

Vytória Piscitelli Cavalcanti [a,*], Adão Felipe dos Santos [a], Filipe Almendagna Rodrigues [a], Willian César Terra [b], Ronilson Carlos Araújo [a], Clerio Rodrigues Ribeiro [a], Vicente Paulo Campos [b], Everlon Cid Rigobelo [c], Flávio Henrique Vasconcelos Medeiros [b], Joyce Dória [a]

[a] Department of Agriculture, Federal University of Lavras (UFLA), P.O. Box: 3037, 37.200-900, Lavras, MG, Brazil
[b] Department of Phytopathology, Federal University of Lavras (UFLA), P.O. Box: 3037, 37.200-900, Lavras, MG, Brazil
[c] Department of Plant Production, University of São Paulo State (UNESP), 14884-900 Jaboticabal, SP, Brazil

ARTICLE INFO

Keywords:
Lactuca sativa
Meloidogyne incognita
Bacillus subtilis
Biological control
Vegetation index
Vegetation cover

ABSTRACT

Lettuce (*Lactuca sativa*) is an important horticultural commodity all over the world, and its growth can be affected by root-knot nematodes (*Meloidogyne* spp.). To keep track of plant behaviors, growers are using new technologies. In this paper, aerial images were obtained using a low-cost unmanned aerial vehicle (UAV) to gather crop information in a short time giving acceptable accuracy for decision-making in the field. Evaluations were done to check the flight height interference in the image's quality for lettuce mapping, and select the best one to estimate the effect of root-knot nematode incidence on lettuce growth. In a field infested with *M. incognita*, lettuce seedlings were planted in plots treated with bionematicide and control plots. Aerial images were obtained using low-cost UAV in four flight heights performed for five weeks, along with field measurements. Images were processed and used to calculate vegetation indices (VI) and vegetation cover (VC). After lettuce harvesting, nematode eggs were extracted from plants' roots and quantified. Plots treated with bionematicide showed no difference from the control plots in eggs number and lettuce growth. Differences in VI values between the flight heights were not consistent, suggesting that VI values could be affected by the lack of luminosity calibration in each flight condition. VC values calculated from field data presented strong positive correlations with VI and VC values from UAV image data, indicating that RGB images obtained by UAV can be used in the detection of diseases that affect plant growth, as well as following up harvesting time.

1. Introduction

The development of tools to reduce the work and time spent monitoring crops in the field have ever aroused great interest of the farmers. The use of aerial images in the management of agricultural fields has been gaining more and more space in the field's routine [1–4]. The images processing provide a lot of data on the crop field in less time consuming than traditional methods [5]. By extracting information from aerial images, it is possible to delineate management zones and build cultivation guidelines according to the needs of plants in each zone. In this way, the farmers are able to check the plants growth and nutritional status, identify the best time to harvest, detect pests and diseases, being prepared to perform the precision agriculture [2,4,6–8].

Aerial images can be obtained from low-cost unmanned aerial vehicles (UAVs), increasing this technology access and, consequently, expanding its use [9]. Currently, large and small horticultural farmers use UAV images to obtain information of their crops in a short time and with acceptable accuracy to decision making in the field [10].

Lettuce (*Lactuca sativa*) is a widely consumed horticultural crop in the world and cultivated in all Brazilian regions. Plant-parasitic nematodes are soil-borne pathogens that feed mainly on the roots of susceptible plants. These phytopathogens cause billionaire losses to farmers

* Corresponding author.
E-mail addresses: vytoriapc27@gmail.com (V.P. Cavalcanti), adao.felipe@ufla.br (A.F. dos Santos), filiperodrigues@ufla.br (F.A. Rodrigues), terranema@gmail.com (W.C. Terra), ronnjkarlos7@gmail.com (R.C. Araújo), clerio.ribeiro@estudante.ufla.br (C.R. Ribeiro), vpcampos@ufla.br (V.P. Campos), everlon.cid@unesp.br (E.C. Rigobelo), flaviomedeiros@ufla.br (F.H.V. Medeiros), joyce.doria@ufla.br (J. Dória).

https://doi.org/10.1016/j.atech.2022.100100
Received 5 July 2022; Accepted 27 July 2022
Available online 28 July 2022

around the world, with root-knot nematodes responsible for most of this damage. Lettuce plants are usually susceptible to root-knot nematodes (*Meloidogyne* spp.). Depending on the nematode population level in the soil, it can cause losses of U$ 23.19 for the cultivation of 2000 lettuce plants [11]. UAV images can be used to investigate nematode-infested areas, as reported for coffee [12–14] and soybeans [15] crops, but not yet used in lettuce crop.

The aerial images quality will depend on several factor, such as the camera resolution, flight height, weather conditions, among others [16]. In horticultural crops, which are usually smaller plants, the resolution is an important factor to detect them. The smaller is the flight height, the higher will be the resolution and, consequently, the higher will be the level of detail obtained in the images [17,18]. Thus, it is necessary to identify flight heights to meet the needs of each culture. Looking to simplify the process of monitoring and detecting the occurrence of root-knot nematodes in lettuce crop as well as the reduction of the losses, in this research the objectives were to evaluate the flight height interference in the images quality for lettuce mapping, and select the best one to estimate the effect of *Meloidogyne* spp. incidence on lettuce growth and development using RGB images obtained from UAVs.

2. Material and methods

2.1. Study area

The experiment was carried out at the Center for Technology Development and Transfer – CDTT from the Federal University of Lavras – UFLA, in the municipality of Ijaci, MG, Brazil (Latitude: 21°9′48.73"S, Longitude: 44°55′2.26"W) (Fig. 1).

In a field naturally infested with *Meloidogyne incognita*, lettuce cultivar Solaris seedlings were planted in 2-meter x 1-meter plots. Twenty-four seedlings were planted per plot. The experiment consisted of some plots with the application of *Bacillus subtilis* BV09 (Biobaci®) and others without applications (control). Four repetitions (plots) were performed for each treatment (T1 = without application, and T2 = *B. subtilis* BV09 application).

2.2. Aerial images acquisition

The aerial images were obtained with a DJI Phantom 4 Advanced aircraft (Fig. 2) with the following characteristics: weight of 1388 g; diagonal size (propellers excluded) of 350 mm; maximum speed of 72 km/h; maximum angle of inclination of 42°, and maximum flight time of 30 min. The GPS/GLONASS positioning system was equipped, by default, with a 1-in. CMOS sensor to capture video (up to 4,096 × 2,160 p at 60 fps) and photos up to 20 megapixels.

The planning was performed using the free software Pix4DCapture installed on an iOS system. The flight plan was defined according to the following characteristics: speed of 3 m/s and all side with 80% overlap. The same mission was applied to four different flight heights: 10 m (GSD = 0.44 cm/pixel), 15 m (GSD = 0.66 cm/pixel), 20 m (GSD = 0.88 cm/pixel), and 25 m (GSD = 1.09 cm/pixel). The flights were performed weekly for five weeks, beginning two weeks after lettuce planting until three days before harvesting.

2.3. Image processing

The images taken from each week were processed using Agisoft PhotoScan software (Agisoft LLC, St. Petersburg, Russia). This software, based on a SfM algorithm, is superior to others in terms of precision. Also, the software provides three-dimensional points and produces a reliable data set to create dense point clouds. The input photographs can then be mosaicked and orthorectified to create the DEM by converting the point clouds into vector mesh or raster digital elevation models (DEMs) [19].

To generate the orthomosaic, the images were alignment using the photo-triangulation process and generation of a sparse point cloud, which defined the coordinate system of the terrain (Step 1). In sequence, the sparse point cloud generated in the previous step was densified providing more detailed representation of the mapped area and was also referenced the WGS 84 Zone 23S local coordinate system (Step 2). In step 3, a model was built that accurately represented the three-dimensional mapped terrain. Thus, it was possible to represent the digital surface model (DSM), and, after filtering the point cloud of the

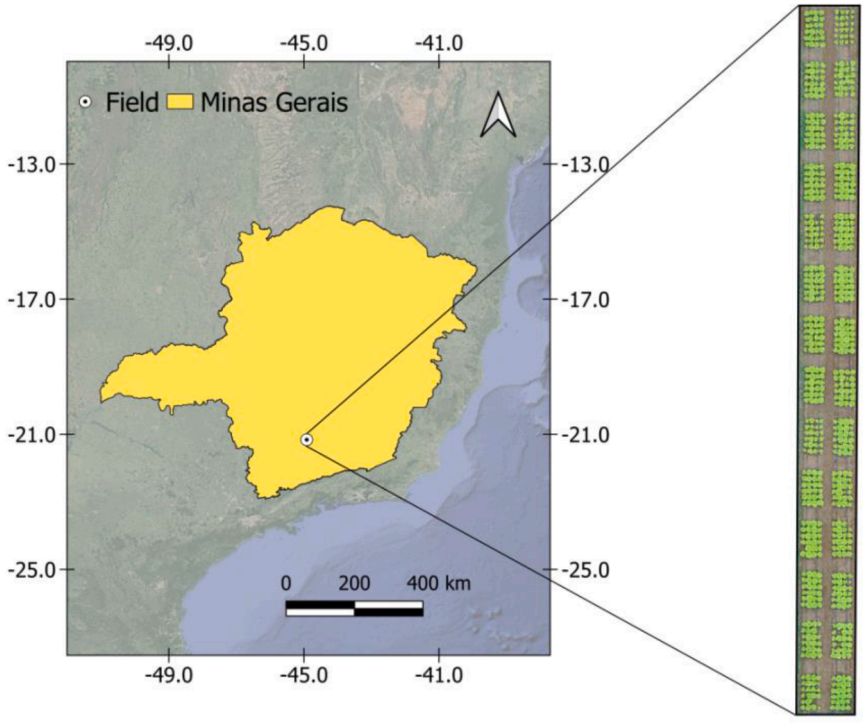

Fig. 1. Location of the experimental area.

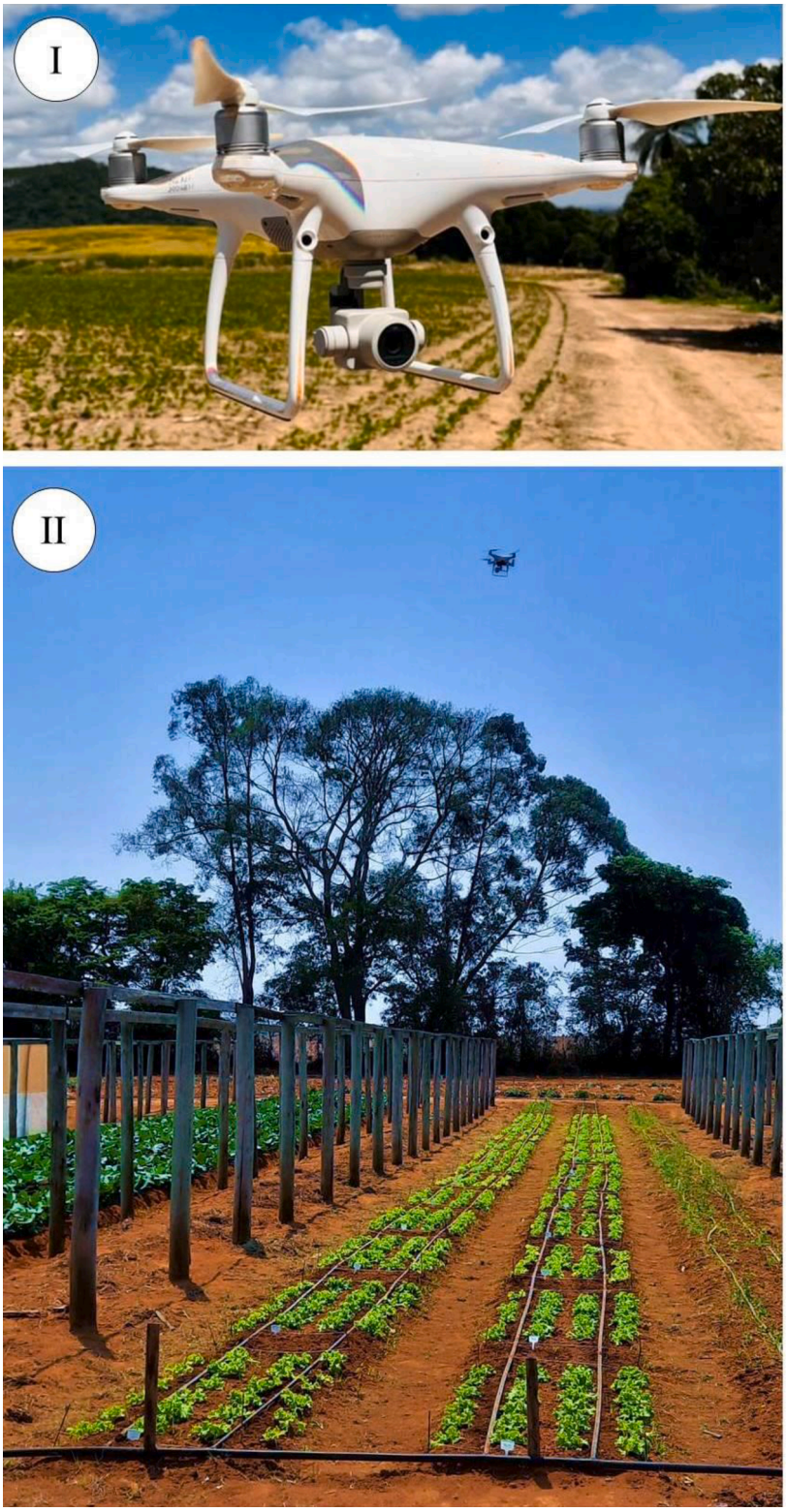

Fig. 2. DJI Phantom 4 Advanced aircraft (I) and image capturing process (II).

soil, it was possible to visualize the digital terrain model (DTM). In step 4, the texture was applied to the model obtained in the previous step to improve the visual appearance and distinction between objects. Step 5 consisted of the creation of the DEM. The generated products were two-dimensional raster format representations of the DSM and DTM. Lastly, the orthomosaic was generated in step 6.

Despite the low-cost UAVs produce georeferenced images (geotags), it is necessary to adjust the actual positioning with the aid of Ground Control Point (GCP) to improve the accuracy, especially when you are working with different flight days [20]. The Qgis software (QGIS Development Team, Open Source Geospatial Foundation) was used to identify and align the coordinates in all orthomosaic.

2.4. Vegetation cover calculation

The process used to calculate the vegetation cover was performed in QGIS starting by removing the soil, as described below and summarized in Fig. 3.

After the align images (orthomosaic image), the soil reflectance was removed from plant reflectance, in Qgis software, as follows: a shapefile was created for soil and plant classes (step 1) and a vegetation index was calculated (step 2). In step 3, soil and vegetation were segmented through the images classification with training using the supervisor classification plug-in in QGIS dzetsaka: Classification tool (https://github.com/nkarasiak/dzetsaka/). Then, the file with soil and vegetation segmented was poligonyzed, from raster to vector (step 4). In vector symbology, the options 'classify categorized' and 'enable editing' were selected to disable vegetation, select soil and delete all soil portion, remaining only vegetation portion (step 5). In geoprocessing tools, the option 'Buffer(s)' was selected to change the distance from 10 to 0.0001 meters (step 6). In step 7, the vegetation index raster file was cliped by the mask layer using the vectorized file with only vegetation portion, finally obtaining the file with only plant reflectance.

After removing the soil, zonal statistics was used to count the number of pixels of each plot, i.e., the number of pixels of the vegetable portion (step 8). Finally, using the field calculator, the vegetation cover was calculated through the following equation (step 9):

$$\text{Vegetation cover} = \frac{((\text{pixel count in plot} \times \text{pixel size}) \times 100)}{\text{plot area}} \quad (1)$$

2.5. Vegetation index calculation

Vegetation indices based on the visible portion of the electromagnetic radiation spectrum were calculated using QGIS. The green leaf index (GLI) and excess green vegetation index (ExG) can be used to separate vegetation and soil portions in the images [21]. The vegetation indices and their equation were described in Table 1.

2.6. Field measurements

The diameter of four lettuce plants per plot was measured in the field weekly for five weeks, on the same dates as the flights. Vegetation cover from the field data was obtained by calculating the mean area per plant of the measured plants and, then, multiplying by the total number of plants in the plot area.

After harvesting, the roots were washed and the *M. incognita* eggs were extracted. The number of eggs is a parameter generally used to assess the nematode population in the soil, and is directly related to the damage caused by this pathogen. To remove the eggs from the root system, the roots of each plant were cut and shaken in a blender using NaOCl at a concentration of 0.5% for 40 s. Next, the eggs were collected in a sieve of 0.025 mm opening (500 mesh), rinsed in running water and stored in plastic tubes in the refrigerator [23]. Then the eggs suspension was cleaned following the technique described by Jenkins [24]. After this process the eggs were counted in a stereoscopic microscope using a Peters' camera, and done 3 times per plot.

Table 1
Vegetation indices based on the visible portion of the electromagnetic radiation spectrum and their equation.

Vegetation index	Equation	Reference
Green Leaf Index (GLI)	$GLI = \frac{(2 \times green - red - blue)}{(2 \times green + red + blue)}$	[21]
Excess Green Vegetation Index (ExG)	$ExG = 2 \times green - red - blue$	[22]

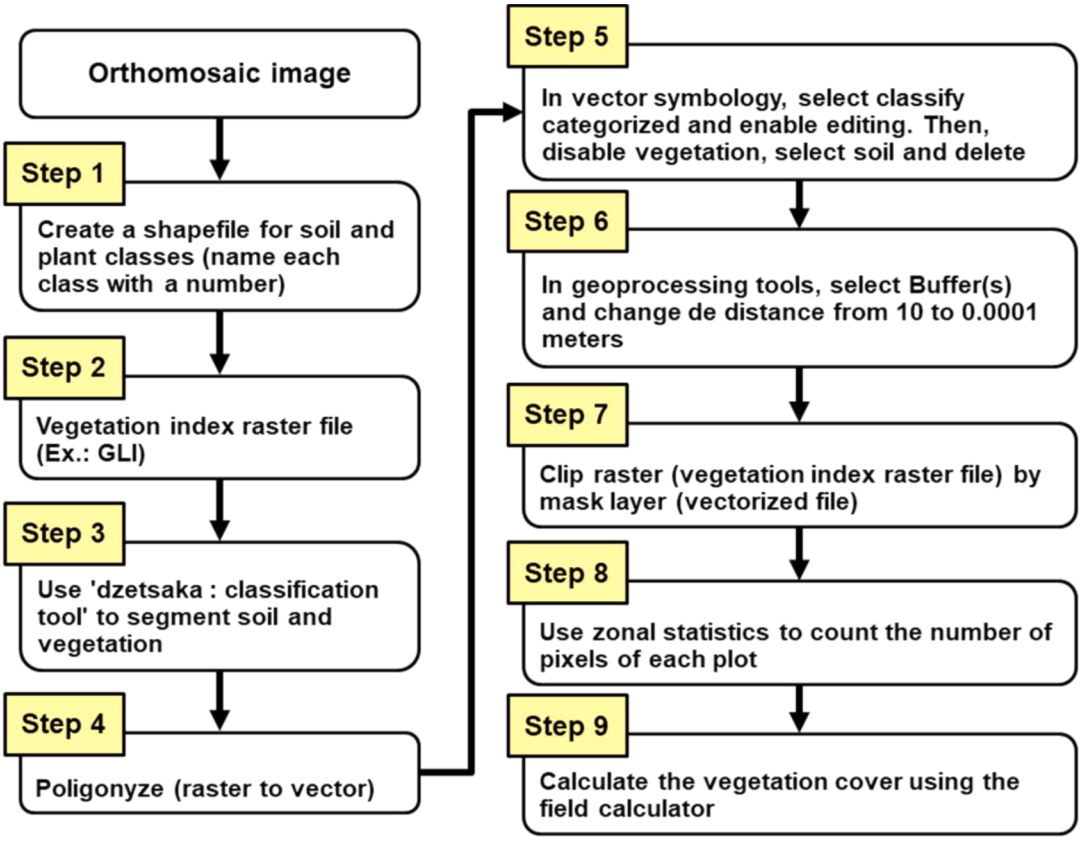

Fig. 3. The summarized processes to remove the soil for vegetation cover calculation in QGIS.

2.7. Data analysis

Shapiro–Wilk test was used to determine the normality of the data and Levene's test was performed to assess the homogeneity of variance. Then, the data were submitted to analysis of variance (ANOVA). The vegetation cover and vegetation indices means were compared by Tukey's test for each day after planting, and the eggs number and root fresh mass means were compared by Student's t test. Analysis of variance was performed to verify if there is an effect of the time on the lettuce growth, measured through the values of vegetation cover obtained from UAV image data and from field data. In addition, to assess the influence of time on lettuce growth, regression models were fitted. Pearson correlation analysis was conducted to detect the relationship between the variables, and the linear regression was conducted to describe the relationship between them. The statistical analyses were carried out by using R software [25].

3. Results

3.1. Comparing flight heights

The visible spectrum images captured in four different heights (10, 15, 20, and 25 m) show losses in image resolution as the flight height increases which reduces the details captured in the image, especially at 25 m height (Fig. 4, I). The resolution losses as the flight height increased were also observed when the vegetation indices (ExG and GLI) were calculated (Fig. 4, II and III).

Regardless of the loss of resolution as the flight height increases, significant differences were not found for the vegetation cover calculated from the aerial images obtained from 10, 15, 20, and 25 m height (Table 2). The vegetation cover calculated from the aerial images and from field data showed similar results, except at 30 DAP when the field data showed high variation, alerting to possible failures in field data collection (Table 2).

Table 2
Vegetation cover calculated from different flight heights and from field data of lettuce plants on different days after planting.

Vegetation cover Flight Heights:	15 DAP	30 DAP	44 DAP
10 m	22.569 ± 3.685 a	89.568 ± 6.055 a	95.653 ± 4.964 a
15 m	24.436 ± 3.821 a	89.581 ± 5.674 a	96.187 ± 4.448 a
20 m	25.271 ± 4.430 a	90.400 ± 5.144 a	95.922 ± 4.562 a
25 m	26.476 ± 4.085 a	91.173 ± 5.186 a	96.395 ± 4.315 a
Field data	23.066 ± 6.437 a	74.856 ± 16.862 b	90.634 ± 11.323 a

*Means followed by the same letter in the columns do not differ by Tukey test ($p<0.05$). *DAP – days after planting.*

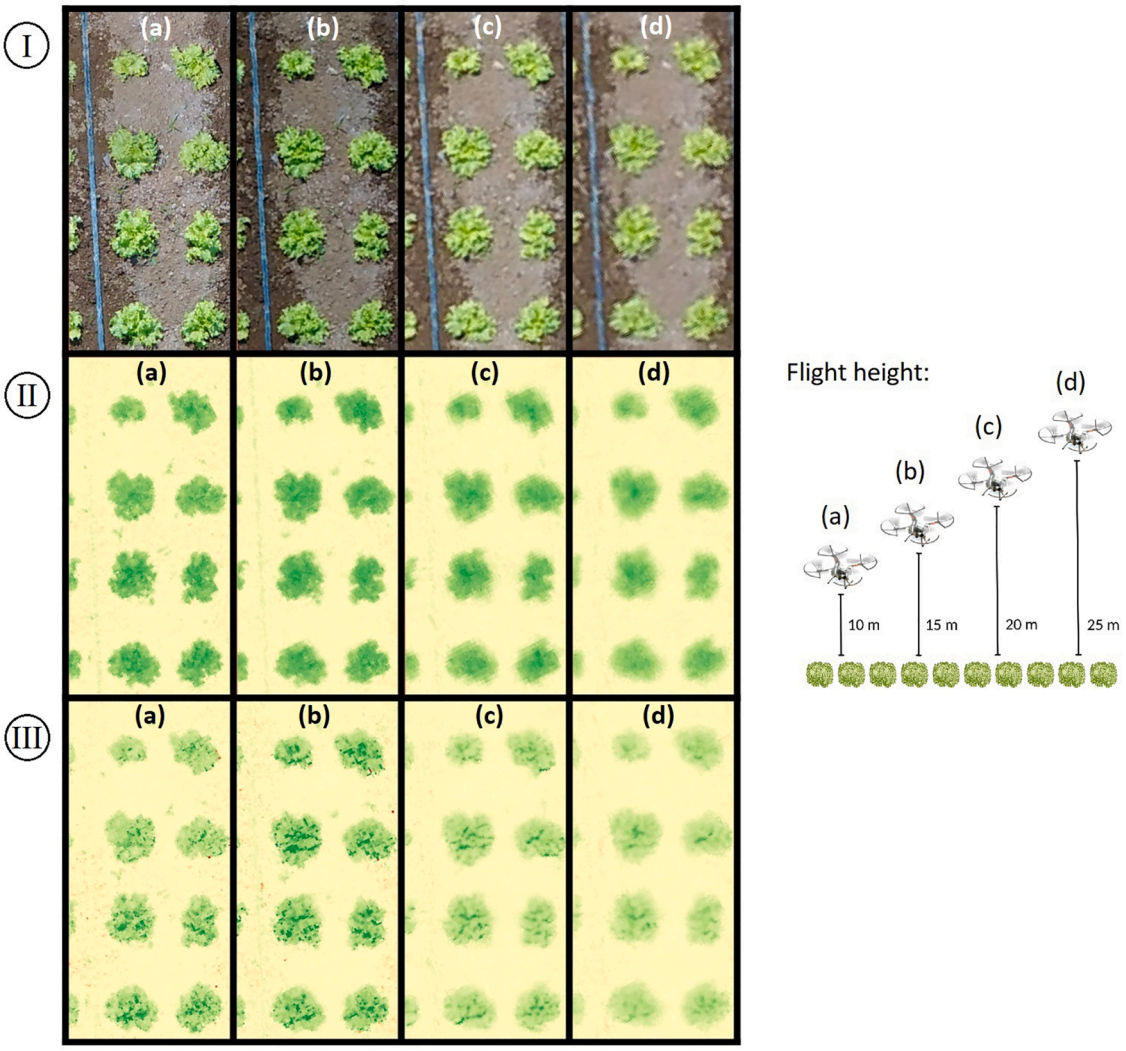

Fig. 4. Visible spectrum images (I), from lettuce plants with 15 days after planting, captured in four different heights (10 m (a), 15 m (b), 20 m (c), and 25 m (d)), and images from the vegetation indices: Excess Green Vegetation Index – ExG (II) and Green Leaf Index – GLI (III). Scale: 1:10.

The vegetation index values were compared in the different flight heights, for each evaluation day (days after planting) (Table 3). For GLI there was a significant difference ($p < 0.05$) between the flight heights in most of the days, except at 23 days after planting (DAP). At 15 DAP, the best height was 15 m, but at 23 DAP there were no differences between GLI values for all flight heights. At 30 DAP, 15, 20 and 25 m heights were better than 10 m, but at 37 DAP 10 m was the best flight height and the GLI values were decreasing when the height was increasing. In this way, the differences found in GLI values of the flight heights were not consistent over time.

For the ExG, there was no significant difference between the flight heights ($p > 0.05$) over time. Except in 37 DAP, where 10, 15 and 20 m flight heights were better than 25 m (Table 3).

3.2. Lettuce growth over time

The vegetation cover was used to evaluate the lettuce growth over time. There was no significant difference ($p > 0.05$) between the treatments without application (T1) and with the application of *Bacillus subtilis* (T2) for vegetation cover from UAV images data and from field data.

Significant differences were observed in the vegetation cover from UAV images data over time, being fitted a quadratic regression model ($p < 0.01$, $R^2 = 0.9617$, RMSE = 5.481) (Fig. 5). Using data of lettuce diameter collected manually (field data), was possible to estimate a vegetation cover by calculating the area of each plant and multiplying by the number of plants in each plots. For this vegetation cover obtained from the data collected manually in the field, it was also fitted a quadratic regression model ($p < 0.01$, $R^2 = 0.835$, RMSE = 11.758) (Fig. 5). The use of UAV images data to calculate the vegetation cover improved the accuracy in 46.61% when compared to the field measurements.

Vegetation cover values which were calculated from UAV image data and from field data presented strong and positive Pearson correlation ($r = 0.936$, $p < 0.01$). This result indicates that the aerial images obtained from UAV can be used to evaluate the lettuce growth over time, being faster and easier than going to the field and measuring plant by plant.

The GLI presented strong positive Pearson correlation with the vegetation cover values calculated from UAV image data ($r = 0.944$, $p < 0.01$), being fitted a linear regression model with reasonable precision ($R^2 = 0.891$) and accuracy (RMSE = 0.026) (Fig. 6, I). This result using UAV image data showed to be more precise than using field data, which showed positive correlation ($r = 0.856$, $p < 0.01$) and fitted a linear regression model with lower precision ($R^2 = 0.732$) (Fig. 6, I).

In contrast, when ExG was used in both types of data collection (UAV image data and field data), the Pearson correlation between vegetation cover values and the vegetation index was strong and positive with values higher than 0.9 (UAV image data: $r = 0.982$, $p < 0.01$; field data: $r = 0.936$, $p < 0.01$). In additional, the regression models had similar performance with $R^2 = 0.965$ and $R^2 = 0.877$ for UAV image and field data, respectively (Fig. 6, II).

Table 3
Vegetation indices (GLI and ExG) of lettuce plants calculated from 10, 15, 20, and 25 m flight height on different days after planting (DAP).

GLI Flight Heights:	15 DAP	23 DAP	30 DAP	37 DAP	44 DAP
10 m	0.048 ± 0.011 b	0.175 ± 0.030 a	0.240 ± 0.027 b	0.289 ± 0.022 a	0.202 ± 0.023 ab
15 m	0.061 ± 0.014 a	0.173 ± 0.028 a	0.259 ± 0.029 a	0.273 ± 0.018 b	0.211 ± 0.022 a
20 m	0.042 ± 0.013 b	0.159 ± 0.030 a	0.251 ± 0.029 ab	0.244 ± 0.022 c	0.182 ± 0.022 b
25 m	0.044 ± 0.009 b	0.166 ± 0.029 a	0.252 ± 0.031 ab	0.221 ± 0.024 d	0.184 ± 0.023 b
ExG Flight Heights:	**15 DAP**	**23 DAP**	**30 DAP**	**37 DAP**	**44 DAP**
10 m	27.339 ± 5.755 a	81.753 ± 11.046 a	136.640 ± 14.732 a	152.647 ± 11.776 a	128.837 ± 12.457 a
15 m	28.802 ± 6.044 a	81.832 ± 12.248 a	137.582 ± 14.727 a	149.790 ± 11.060 a	130.664 ± 11.158 a
20 m	25.269 ± 6.966 a	78.783 ± 12.058 a	135.442 ± 13.919 a	147.545 ± 12.583 ab	124.660 ± 11.901 a
25 m	26.070 ± 5.337 a	86.161 ± 13.218 a	133.238 ± 13.814 a	142.244 ± 13.016 b	124.689 ± 11.537 a

*Means followed by the same letter in the columns do not differ by Tukey test ($p<0.05$). *DAP – days after planting; GLI – Green Leaf Index; ExG – Excess Green Vegetation Index*.

3.3. Root-knot nematode in lettuce roots

Root-knot nematode population was evaluated by the quantification of the number of eggs in lettuce roots (Table 4). Although lettuce plants with the application of B. subtilis BV09 (T2) presented less number of eggs in their roots, significant differences ($p > 0.05$) were not found between the treatments with and without application of *B. subtilis* BV09 (Table 4). The absence of a significant difference in the number of eggs between the treatment with the application of *B. subtilis* BV09 (T2) and the control treatment (T1) may be related to the high variation in the number of eggs in lettuce roots of T1 (Table 4). It may have happened due to the lack of standardization of the initial inoculum, as the experiment was carried out in an environment with natural infestation.

There was no significant difference ($p > 0.05$) between the treatments for fresh root biomass (Table 4), and the appearance of the roots and shoots were similar among plants with and without *B. subtilis* BV09 application (Fig. 7, I and II).

4. Discussion

Using a low-cost UAV, DJI Phantom 4 Advanced aircraft, it was possible to satisfactorily calculate the vegetation cover of a lettuce plantation and differentiate lettuce plant growth between the assessment days. RGB images have been used in many research to segment plants from the overall image [26–28], providing data to calculate the vegetation cover. In this research, it was possible to calculate lettuce vegetation cover in the field through RGB images, with consistent results with the field measurements.

The vegetation indices GLI and ExG values, which presented strong positive correlation with the vegetation cover calculated from UAV and field data, can be used to identify on the maps where plants growth is affected. This technology can be applied in the evaluation of lettuce growth and in the detection of diseases that affect plant growth, such as root-knot nematode, but mainly for diseases that affect the aerial part of the plants such as the tomato spotted wilt virus on lettuce.

In horticultural crops, there are reports of flight height around 15 m for potato [18] and 20 m for lettuce [29], close to the used herein. Thus, the flight height may vary according to the level of needed detail to obtain the information for each crop. Most flight heights between 10 to 25 m are sufficient for evaluations of vegetation indices for lettuce crop.

Observing the vegetation indices values, the differences between the flight heights were not consistent, being not possible to find the best flight height for these parameters. These results lead to the conclusion that maybe the differences in the vegetation indices value between the flight heights were not caused by the flight height. Probably, these differences in vegetation indices values were caused by another factor, such as the lack of luminosity calibration in every flight. According to Woebbecke et al. [22], the excess green vegetation index (ExG) works well for both non-shaded and shaded sunlit conditions, which can explain the similarity in the ExG values for the flight heights. It corroborates the hypothesis that the inconsistent variation in the green leaf index (GLI) values can be due to the variation in the weather conditions in each flight.

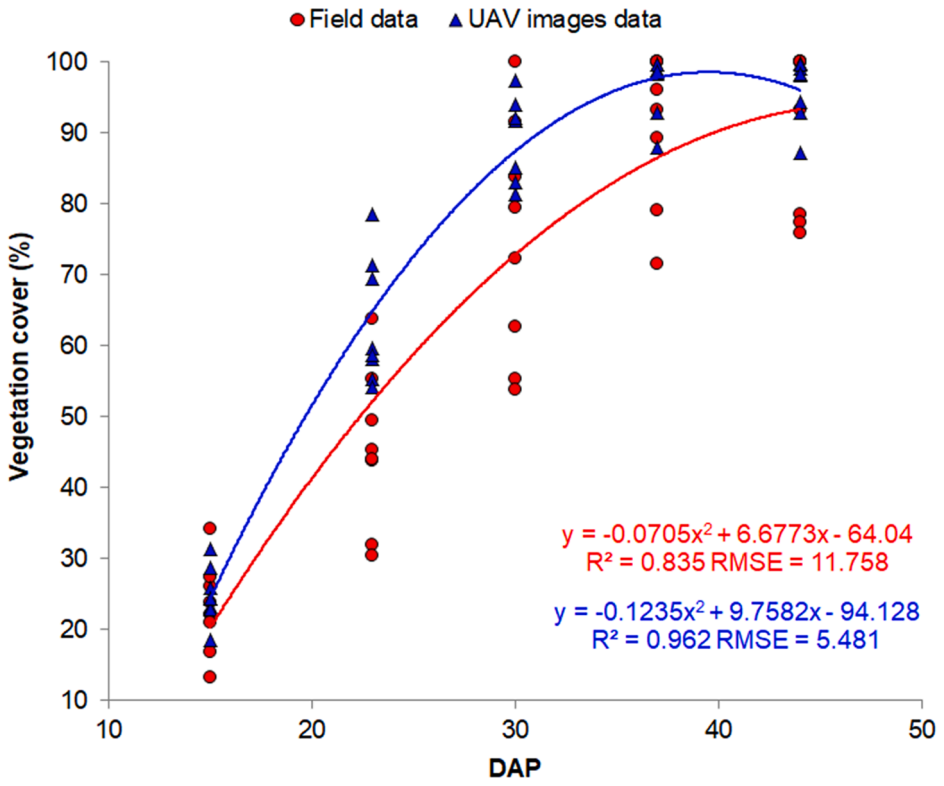

Fig. 5. Regression of lettuce growth measured by the vegetation cover (%) over time (DAP = days after planting), calculated from field data (red circle) and from UAV image data (blue triangle).

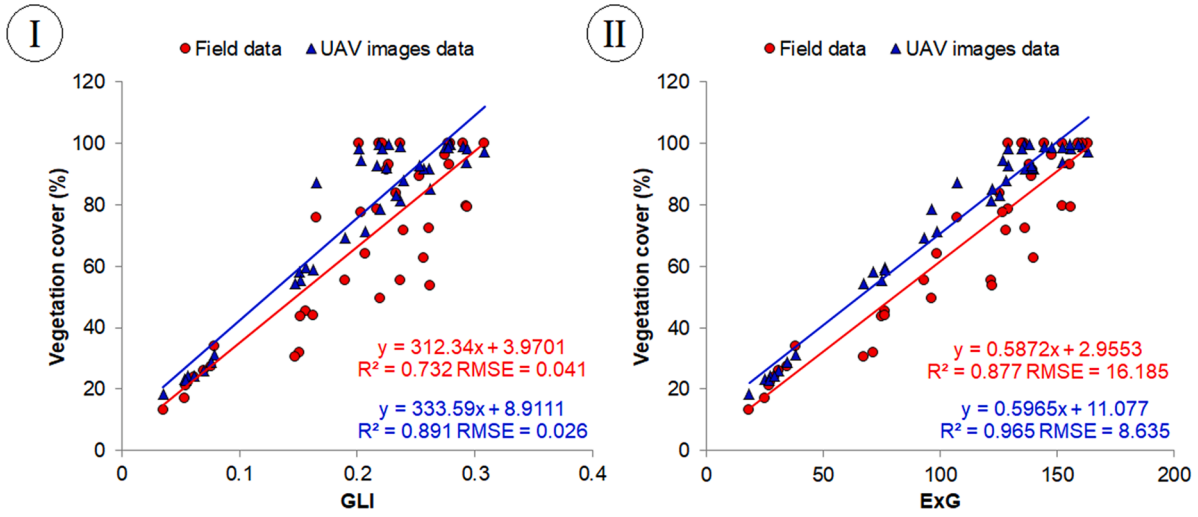

Fig. 6. Regression between the vegetation index values (I = GLI and II = ExG) and the lettuce growth measured by the vegetation cover (%) calculated from field data (red circle) and from UAV image data (blue triangle).

Table 4
Root-knot nematode population in lettuce roots with and without the application of *B. subtilis* BV09.

Treatment	N° of eggs/g of root	Fresh root biomass (g)
T1	55.671 ± 46.265 a	28.333 ± 7.835 a
T2	21.175 ± 13.247 b	32.583 ± 6.744 a

*Means followed by the same letter in the columns do not differ by Student's t-test ($p < 0.05$). *T1 – without the application of B. subtilis BV09 (control); T2 - with the application of B. subtilis BV09.*

Many researchers are using RGB images to calculate vegetation index and estimate nitrogen and chlorophyll content [6,26,30,31]. Some of the researchers who used RGB images to determine nitrogen content and chlorophyll concentration used camera support with standardized light source to capture images in greenhouse tomato [30] and lettuce [32] crops. In the field, however, it is not possible to perform the luminosity standardization in this way to capture RGB images for comparing different flights. Thus, images capture in the field requires calibration of the luminosity through the use of a panel, even before and after a flight, and a multispectral camera with an incidence light sensor [33]. Multispectral cameras have been used to calculate vegetation index and assess

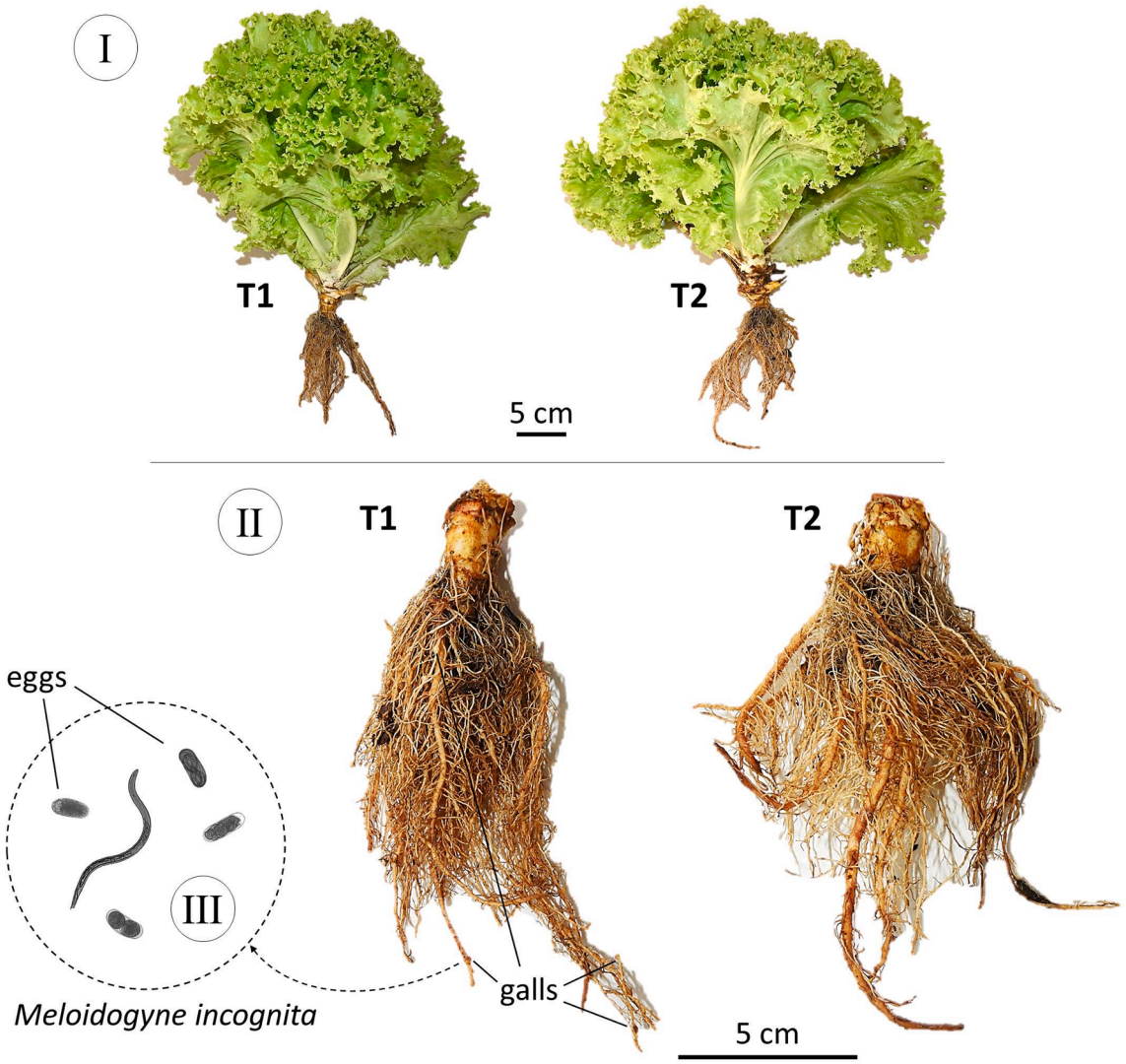

Fig. 7. Representative image of lettuce plants (I) and their roots (II) from the field which was infested with *Meloidogyne incognita*, showing the galls and eggs (III). T1 – without the application of *B. subtilis* BV09 (control); T2 – with the application of *B. subtilis* BV09.

the health of lettuce plants [9,34], as well as hyperspectral cameras have been used to assess lettuce water uptake by measuring leaf water content [35].

In this context, RGB digital cameras have the advantage of being cheaper and presenting a high resolution. However, the use of RGB images has limitations because of the high field luminosity effect and the impossibility of calibrating it, impeding the comparison of field RGB images. The color indices from RGB images can be used mainly to segment plants from the overall image and, as evaluated in this research, the vegetation indices GLI and ExG can be used to detect failures in plant growth and to identify the occurrence of diseases that affect the lettuce plant growth.

Lettuce cultivar Solaris proved to be susceptible to *M. incognita*, as found by Sgorlon et al. [36], presenting *M. incognita* infection with gall formation and eggs production (Fig. 7 and Table 4). On the other hand, this cultivar showed some tolerance to *M. incognita*, being not observed damage to lettuce plant growth.

5. Conclusions

Vegetation indices and vegetation cover calculation using RGB images captured by a low-cost UAV proved to be efficient in measuring lettuce growth over time with higher accuracy than field measurements. This technology can be applied for the detection of diseases that affect lettuce growth, as well as help following up harvesting time. RGB images should not be used to compare vegetation index values between different flights, since the light incidence and cloudiness can affect the vegetation index performance. In this case, multispectral or hyperspectral images are more suitable. Future research can assess the use of proximal sensors for plant-parasitic nematode detection in lettuce crop.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors extend thanks to CAPES for granting doctorate's scholarships, to the Federal University of Lavras (UFLA) and Technology Development and Transfer Center (CDTT) for the structure provided to carry out the experiments and for the support of the technicians and professors. Thanks to the institutions: FAPEMIG, CNPq, and CAPES for providing the necessary equipment and technical support for the experiments. Thanks to Grupo Vittia for providing the Biobaci® and

technical support. Thanks to Hugo Nunes Martins Nogueira for assisting in obtaining the UAV images and to Sansão Augusto Germano, Natalia Bernardes Machado, Otávio Bernardes Machado, Regiane Alves Belizario, Kamilly Maria Fernandes Fonseca, Brenda Miriam Silva and Maria Luiza Paiva de Oliveira for assisting in field measurements.

References

[1] V. Choudhary, L. Prasad, Morpho-pathological, genetic variations and population structure of *Sclerotinia sclerotiorum*, Int. J. Plant Res. 25 (2012) 178–183.
[2] E. Raparelli, S. Bajocco, A bibliometric analysis on the use of unmanned aerial vehicles in agricultural and forestry studies, Int. J. Remote Sens. 40 (2019) 9070–9083, https://doi.org/10.1080/01431161.2019.1569793.
[3] U.R. Mogili, B.B.V.L. Deepak, Review on application of drone systems in precision agriculture, Proc. Comput. Sci. 133 (2018) 502–509, https://doi.org/10.1016/j.procs.2018.07.063.
[4] J. Barbedo, A review on the use of unmanned aerial vehicles and imaging sensors for monitoring and assessing plant stresses, Drones 3 (2019) 40, https://doi.org/10.3390/drones3020040.
[5] A. Vibhute, S.K. Bodhe, Applications of image processing in agriculture: a survey, Int. J. Comput. Appl. 52 (2012) 34–40, https://doi.org/10.5120/8176-1495.
[6] Y. Fu, G. Yang, Z. Li, X. Song, Z. Li, X. Xu, P. Wang, C. Zhao, Winter wheat nitrogen status estimation using UAV-based RGB imagery and gaussian processes regression, Remote Sens. 12 (2020) 3778, https://doi.org/10.3390/rs12223778.
[7] L. Yubin, D. Xiaoling, Z. Guoliang, Advances in diagnosis of crop diseases, pests and weeds by UAV remote sensing, Smart Agric. 1 (2019) 1–19.
[8] G. Grenzdörffer, A. Engel, B. Teichert, The photogrammetric potential of low-cost UAVs in forestry and agriculture, Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. 31 (2008) 1207–1214.
[9] G. Cucho-Padin, H. Loayza, S. Palacios, M. Balcazar, M. Carbajal, R. Quiroz, Development of low-cost remote sensing tools and methods for supporting smallholder agriculture, Appl. Geomat. 12 (2020) 247–263, https://doi.org/10.1007/s12518-019-00292-5.
[10] B.K. Handique, C. Goswami, C. Gupta, S. Pandit, S. Gogoi, R. Jadi, P. Jena, G. Borah, P.L.N. Raju, Hierarchical classification for assessment of horticultural crops in mixed cropping pattern using uav-borne multi-spectral sensor, Int. Arch. Photogramm. Remote Sens. Spat. Inf. Sci. XLIII-B3-2 (2020) 67–74, https://doi.org/10.5194/isprs-archives-XLIII-B3-2020-67-2020.
[11] L.K.C. Rabello, A. Oliveira Gongalves, T.P. da Cruz, F. Domingo Zinger, W.C. de Jesus Júnior, L. Lagen Rodrigues, A.F. de Souza, W. Bucker Moraes, F. Ramos Alves, Quantification of damage and yield losses caused by Root-knot nematode in lettuce in Brazil, Idesia (Arica) 39 (2021) 121–130, https://doi.org/10.4067/s0718-34292021000200121.
[12] C.A.M. De Abreu Júnior, G.P. Vinhal, L.C. Moura Xavier, G.D. Martins, B.S. Vieira, Mapeamento de nematoides na cultura cafeeira a partir de imagens multiespectrais obtidas por aeronaves remotamente pilotadas, Caminhos Geogr. 21 (2020), https://doi.org/10.14393/RCG217651255.
[13] A.J. Oliveira, G.A. Assis, E.R. Faria, J.R. Souza, K.C.T. Vivaldini, V. Guizilini, F. Ramos, C.T.C. Mendes, D.F. Wolf, Analysis of nematodes in coffee crops at different altitudes using aerial images, in: 2019 27th European Signal Processing Conference, IEEE, 2019, pp. 1–5, https://doi.org/10.23919/EUSIPCO.2019.8902734.
[14] A.J. Oliveira, G.A. Assis, V. Guizilini, E.R. Faria, J.R. Souza, Segmenting and detecting nematode in coffee crops using aerial images (Eds.), in: D. Tzovaras, D. Giakoumis, M. Vincze, A. Argyros (Eds.), Computer Vision Systems ICVS 2019. Lecture Notes in Computer Science, Springer, Cham., 2019, pp. 274–283, https://doi.org/10.1007/978-3-030-34995-0_25.
[15] B.H.T. Arantes, V.H. Moraes, A.M. Geraldine, T.M. Alves, A.M. Albert, G.J. da Silva, G. Castoldi, Spectral detection of nematodes in soybean at flowering growth stage using unmanned aerial vehicles, Ciência Rural 51 (2021), https://doi.org/10.1590/0103-8478cr20200283.
[16] T. Berteška, B. Ruzgienė, Photogrammetric mapping based on UAV imagery, Geod. Cartogr. 39 (2013) 158–163, https://doi.org/10.3846/20296991.2013.859781.
[17] J. Peña, J. Torres-Sánchez, A. Serrano-Pérez, A. de Castro, F. López-Granados, Quantifying efficacy and limits of unmanned aerial vehicle (UAV) technology for weed seedling detection as affected by sensor resolution, Sensors 15 (2015) 5609–5626, https://doi.org/10.3390/s150305609.
[18] S. Sankaran, L.R. Khot, C.Z. Espinoza, S. Jarolmasjed, V.R. Sathuvalli, G. J. Vandemark, P.N. Miklas, A.H. Carter, M.O. Pumphrey, N.R. Knowles, M. J. Pavek, Low-altitude, high-resolution aerial imaging systems for row and field crop phenotyping: a review, Eur. J. Agron. 70 (2015) 112–123, https://doi.org/10.1016/j.eja.2015.07.004.
[19] J.T. Dietrich, Riverscape mapping with helicopter-based structure-from-motion photogrammetry, Geomorphology 252 (2016) 144–157, https://doi.org/10.1016/j.geomorph.2015.05.008.
[20] D. Gómez-Candón, A.I. De Castro, F. López-Granados, Assessing the accuracy of mosaics from unmanned aerial vehicle (UAV) imagery for precision agriculture purposes in wheat, Precis. Agric. 15 (2014) 44–56, https://doi.org/10.1007/s11119-013-9335-4.
[21] F.Z. Bassine, A. Errami, M. Khaldoun, Vegetation recognition based on UAV image color index, in: 2019 IEEE International Conference on Environment and Electrical Engineering 2019 IEEE Ind, Commercial Power Systems Europe (EEEIC /I&CPS Eur., IEEE, 2019, pp. 1–4, https://doi.org/10.1109/EEEIC.2019.8783830.
[22] D.M. Woebbecke, G.E. Meyer, K. Von Bargen, D.A. Mortensen, Color indices for weed identification under various soil, residue, and lighting conditions, Trans. ASAE 38 (1995) 259–269, https://doi.org/10.13031/2013.27838.
[23] R.S. Hussey, K.R. Barker, A comparison of methods for colecting inocula of meloidogyne spp including a new technique, Plant Dis. Rep. 57 (1973) 1025–1028.
[24] W.R. Jenkins, A rapid centrifugal-flotation technique for separating nematodes from soil, Plant Dis. Rep. 48 (1964) 692.
[25] R Core Team, R: A language and environment for statistical computing, (2019). https://www.r-project.org/.
[26] J. Torres-Sánchez, J.M. Peña, A.I. de Castro, F. López-Granados, Multi-temporal mapping of the vegetation fraction in early-season wheat fields using images from UAV, Comput. Electron. Agric. 103 (2014) 104–113, https://doi.org/10.1016/j.compag.2014.02.009.
[27] J. Kim, S. Kang, B. Seo, A. Narantsetseg, Y. Han, Estimating fractional green vegetation cover of Mongolian grasslands using digital camera images and MODIS satellite vegetation indices, GIScience Remote Sens. 57 (2020) 49–59, https://doi.org/10.1080/15481603.2019.1662166.
[28] D.G. Fernández-Pacheco, D. Escarabajal-Henarejos, A. Ruiz-Canales, J. Conesa, J. M. Molina-Martínez, A digital image-processing-based method for determining the crop coefficient of lettuce crops in the southeast of Spain, Biosyst. Eng. 117 (2014) 23–34, https://doi.org/10.1016/j.biosystemseng.2013.07.014.
[29] G.M. Maciel, R.B. de A. Gallis, R.L. Barbosa, L.M. Pereira, A.C.S. Siquieroli, J. Vitória Miranda Peixoto, Image phenotyping of inbred red lettuce lines with genetic diversity regarding carotenoid levels, Int. J. Appl. Earth Obs. Geoinf. 81 (2019) 154–160, https://doi.org/10.1016/j.jag.2019.05.016.
[30] A. Mercado-Luna, E. Rico-García, A. Lara-Herrera, G. Soto-Zarazúa, R. Ocampo-Velázquez, R. Guevara-González, G. Herrera-Ruiz, I. Torres-Pacheco, Nitrogen determination on tomato (Lycopersicon esculentum Mill.) seedlings by colour image analysis (RGB), Afr. J. Biotechnol. 9 (2010) 5326–5332. https://www.ajol.info/index.php/ajb/article/view/92074/81517.
[31] M.M. Saberioon, M.S.M. Amin, A.R. Anuar, A. Gholizadeh, A. Wayayok, S. Khairunniza-Bejo, Assessment of rice leaf chlorophyll content using visible bands at different growth stages at both the leaf and canopy scale, Int. J. Appl. Earth Obs. Geoinf. 32 (2014) 35–45, https://doi.org/10.1016/j.jag.2014.03.018.
[32] M.S. Odabas, H. Simsek, C.W. Lee, İ. İseri, Multilayer perceptron neural network approach to estimate chlorophyll concentration index of lettuce (Lactuca sativa L, Commun. Soil Sci. Plant Anal. 48 (2017) 162–169, https://doi.org/10.1080/00103624.2016.1253726.
[33] E.R. Hunt, C.S.T. Daughtry, What good are unmanned aircraft systems for agricultural remote sensing and precision agriculture? Int. J. Remote Sens. 39 (2018) 5345–5376, https://doi.org/10.1080/01431161.2017.1410300.
[34] D.D.W. Ren, S. Tripathi, L.K.B. Li, Low-cost multispectral imaging for remote sensing of lettuce health, J. Appl. Remote Sens. 11 (2017), 016006, https://doi.org/10.1117/1.JRS.11.016006.
[35] R.J. Murphy, B. Whelan, A. Chlingaryan, S. Sukkarieh, Quantifying leaf-scale variations in water absorption in lettuce from hyperspectral imagery: a laboratory study with implications for measuring leaf water content in the context of precision agriculture, Precis. Agric. 20 (2019) 767–787, https://doi.org/10.1007/s11119-018-9610-5.
[36] L.F.F. Sgorlon, E.H.C. Silva, R.S. Soares, H.O. Borges, G.M.M. Diniz, L.T. Braz, P.L. M. Soares, Host status of crispy-leaf lettuce cultivars to root-knot nematodes, Biosci. J. 34 (2018) 1319–1325, https://doi.org/10.14393/BJ-v34n5a2018-39387.

CARTILHA DE ORIENTAÇÃO PARA OS PRODUTORES

Manejo integrado de nematoide-das-galhas na cultura da alface

1. O que são nematoides? E o que são nematoides-das-galhas?

Nematoides são **animais** de tamanhos variados naturalmente **presentes na água, no solo e em organismos vivos**. No solo são encontrados nematoides com **diversos hábitos alimentares** (Figura 1). Estes desempenham importante **papel na ciclagem de nutrientes no solo**.

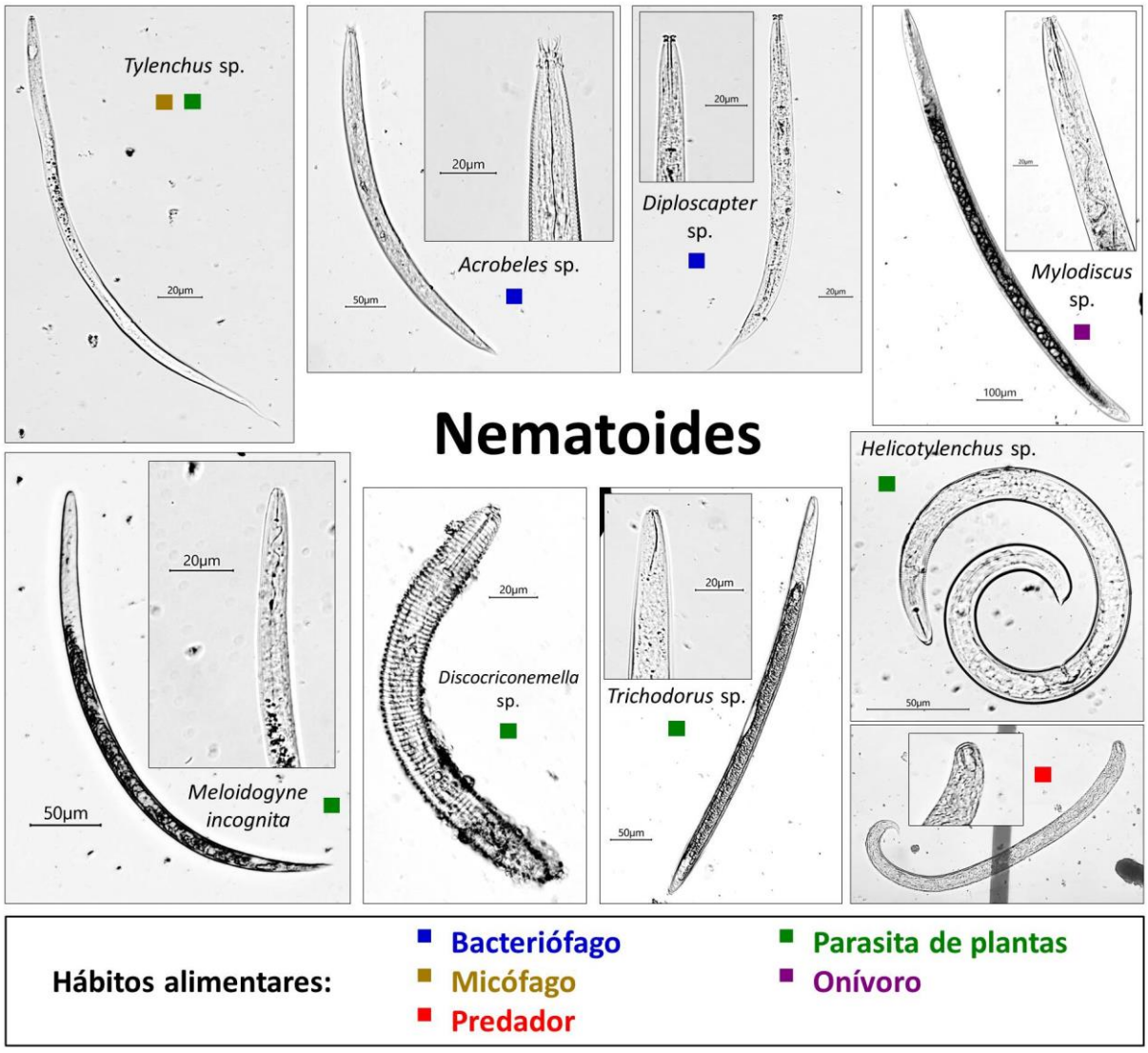

Figura 1. Exemplos de nematoides encontrados no solo (amostra coletada no CDTT-UFLA em Ijaci, MG). **Bacteriófago:** se alimenta de bactérias; **Micófago:** se alimenta de fungos; **Parasita de plantas:** se alimenta nos tecidos de plantas vivas; **Predador:** ingere nematoides, protozoários e outros microinvertebrados; **Onívoro:** pode se comportar como bacteriófago, micófago, parasita de planta e predador.

Os nematoides que se alimentam em tecidos de plantas, conhecidos como **nematoides parasitas de plantas**, podem causar **doenças nas plantas**. Existem diversos tipos de nematoides parasitas de plantas, sendo um deles conhecido como **nematoide-das-galhas** (*Meloidogyne* spp.). Ele entra nas raízes das plantas e provoca a **formação de galhas** (crescimento exagerado de células, como um "tumor") onde ficam se alimentando. A reprodução de um nematoide parasita de plantas pode ser avaliada pelo calculo do **fator reprodutivo (FR)**, que corresponde ao valor da população final do nematoide divido pela sua população inicial (FR = população final / população inicial). Quando o nematoide parasita de plantas consegue se reproduzir no sistema radicular de uma planta com **fator reprodutivo maior que 1** (ou seja, quando a população final do nematoide é maior do que a sua população inicial) esta planta é chamada de **hospedeira** e o seu cultivo provoca aumento da população do nematoide parasita de plantas no ambiente.

Na natureza o nematoide-das-galhas faz **parte do ecossistema**, realizando o seu papel na ciclagem de nutrientes. Porém, quando é feito o **monocultivo com plantas hospedeiras**, como é o caso da alface (*Lactuca sativa*), o nematoide-das-galhas se reproduz descontroladamente até atingir **níveis populacionais em que causa danos** a essa cultura (Figura 2), provocando **prejuízos econômicos aos produtores** com perdas que podem ultrapassar 80% da produção. Dessa forma, surge a necessidade do **controle populacional do nematoide parasita de plantas** para que o **equilíbrio seja reestabelecido no ambiente**.

Figura 2. Canteiro de alface com plantas afetadas pelo ataque de nematoide-das-galhas (*Meloidogyne incognita*). Nessa foto, a zona demarcada em vermelho mostra a ocorrência da doença em formato de reboleira (área definida e de fácil visualização no campo), em que as plantas apresentam crescimento afetado pela ocorrência do nematoide-das-galhas.

2. Como verificar se tem nematoide-das-galhas em sua área?

1º) Verificar se existem sintomas

No campo normalmente os sintomas ocorrem em **reboleiras** (Figura 2), observando-se falhas no stand e plantas de alface com:

- **Crescimento insatisfatório**: nanismo, cabeças de alface menores, mais leves e folhas mais soltas;
- **Amarelamento das folhas**;
- Formação de **galhas** nas raízes (Figura 3).

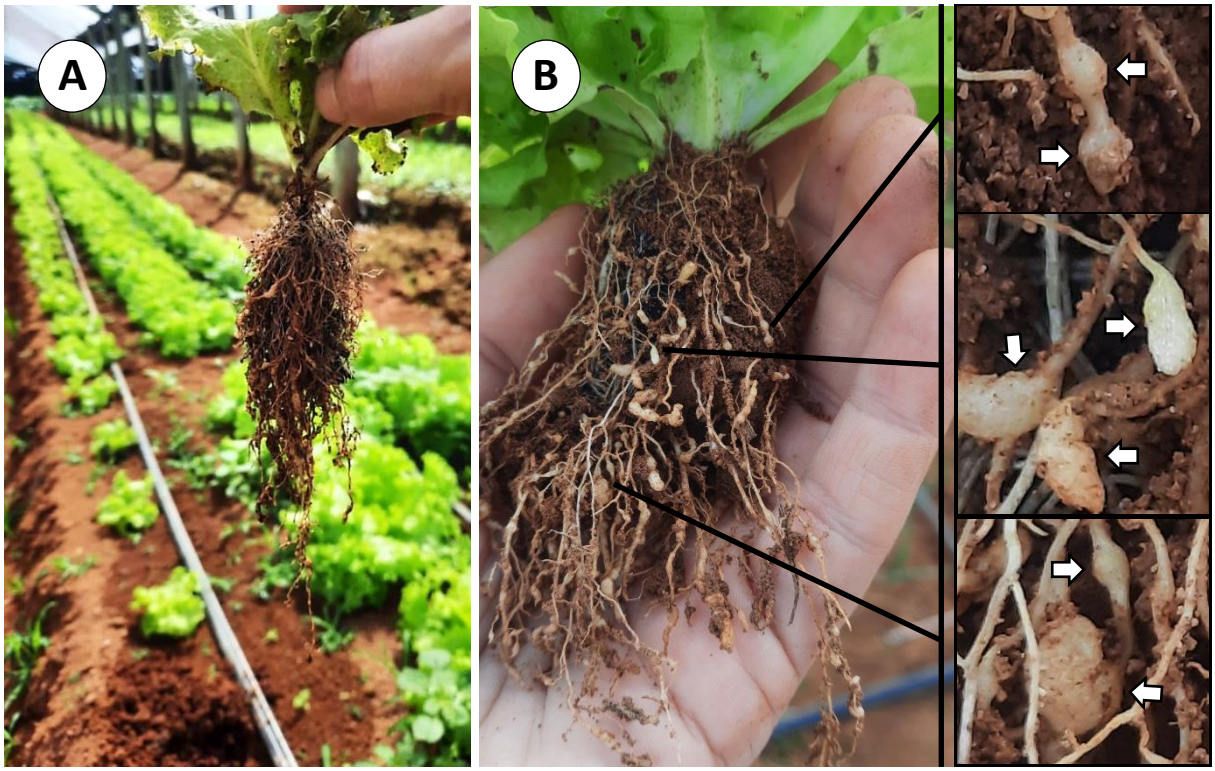

Figura 3. Observação das plantas com crescimento insatisfatório (A) e galhas no sistema radicular (B) causadas pela infecção por nematoide-das-galhas (*Meloidogyne incognita*) nas raízes de alface. As setas brancas estão indicando algumas galhas.

2º) Coletar amostras de solo e raízes

Para confirmar se os sintomas observados estão sendo causados por nematoide-das-galhas, ou para identificar a espécie do nematoide, é necessário fazer uma amostragem de **solo e raízes** no local em que os sintomas foram observados.

A amostragem deverá ser realizada quando o campo estiver úmido (60% da capacidade de campo), evitando coletar solos secos ou encharcados. O solo deve ser coletado em **profundidade entre 10 e 25 cm** contendo o máximo de raízes possível e devem ser coletadas amostras de **raízes com sintomas** (galhas ou lesões radiculares). Durante a amostragem no campo, lembre-se de **proteger as amostras** da exposição ao sol e temperaturas elevadas.

As amostras de solo devem ser colocadas em **saco plástico** fechado e **devidamente identificadas (nome, endereço, local da amostragem, cultura amostrada e data)**. As amostras de raízes também podem ser colocadas em sacos plásticos e precisam ser identificadas (nome, endereço, local da amostragem, cultura amostrada e data), sendo importante **enviar ao laboratório com as raízes ainda vivas**.

As amostras deverão ser mantidas em local com temperatura entre 8 e 10ºC até o envio ao laboratório de análise. **O envio deve ser feito o mais rápido possível, não ultrapassando três dias da data da coleta**.

3º) Levar as amostras identificadas para um laboratório que oferece o serviço de análise nematológica

No laboratório as suas amostras serão analisadas para **confirmar se tem nematoides parasitas de plantas** e **identificar qual(ais) espécie(s)**. É importante saber quais espécies de nematoides parasitas de plantas estão presentes no local e qual o nível populacional (qual o número de indivíduos) de cada espécie.

Além de laboratórios particulares, o serviço de análises nematológicas também é realizado em laboratórios de universidades públicas e institutos de pesquisa. O **Laboratório de Nematologia (Contato: (35) 3829-1469) do Departamento de Fitopatologia (DFP) da Universidade Federal de Lavras (UFLA)** realiza a caracterização de espécies de nematoides parasitas de plantas em amostras de solos e órgãos vegetais.

3. O que fazer se encontrar nematoide-das-galhas em suas plantas?

Se for confirmada a presença de nematoide-das-galhas em suas plantas é necessário **buscar ajuda de um profissional qualificado** para orientar quais são as melhores medidas de manejo a serem realizadas em sua área. Lembrando que cada caso é um caso.

Como o nematoide-das-galhas é de difícil controle, é sempre recomendado que seja feito o **manejo integrado de nematoides**, utilizando-se mais de uma estratégia para conseguir bons resultados.

4. Manejo integrado de nematoide-das-galhas na cultura da alface

Abaixo estão descritos alguns métodos que podem ser utilizados para o manejo de nematoide-das-galhas na cultura da alface.

4.1 Eliminação das raízes infestadas

Ao realizar a colheita das cabeças de alface é interessante **remover as raízes do solo** e **não deixar as raízes infestadas na sua área produtiva**. Essas raízes podem ser queimadas ou descartadas em algum local em que não haja o cultivo de plantas hospedeiras. Fazendo isso **é possível reduzir aproximadamente 80% da população do nematoide-das-galhas no solo**.

4.2 Rotação ou sucessão de culturas com plantas não hospedeiras ou resistentes

Ao cultivar plantas não hospedeiras (FR = 0) ou resistentes (FR ≤ 1) a **reprodução do nematoide-das-galhas é afetada**, **reduzindo a população deste nematoide no solo** e favorecendo o cultivo de alface em sequência na mesma área.

Rotação e sucessão de culturas são técnicas agrícolas diferentes:

- **Rotação de culturas** caracterizada pela alternância anual de espécies vegetais no mesmo local e na mesma estação de cultivo em anos consecutivos;
- **Sucessão de culturas** é caracterizada pelo cultivo de duas ou mais espécies de plantas em sequência na mesma área de cultivo durante o período de até um ano.

O **alho (*Allium sativum*)** é uma espécie resistente e o seu cultivo mostrou-se eficiente em reduzir a população de *Meloidogyne incognita* para cultivo de alface em sequencia na mesma área. Como o alho é uma cultura de alto valor agregado e que apresenta produção mesmo em área infestada com nematoide-das-galhas, trata-se de uma cultura que permite a obtenção de renda mesmo durante o período de manejo deste nematoide.

O **cravo-de-defunto (*Tagetes* spp.)** e a **crotalária (*Crotalaria* spp.)** também são exemplos de espécies resistentes ao nematoide-das-galhas que podem ser utilizadas na rotação ou sucessão de culturas.

4.3 Consórcio de culturas com plantas não hospedeiras ou resistentes

Ao cultivar a alface em consórcio com plantas não hospedeiras (FR = 0) ou resistentes (FR ≤ 1) é possível **reduzir a população do nematoide-das-galhas na área infestada** e **manter pelo menos uma parte da produção de alface durante o manejo**. Para que os resultados sejam satisfatórios, **é necessário que o ciclo de cultivo da planta não hospedeira ou resistente seja mais longo do que o ciclo de cultivo da alface**.

O consórcio de culturas é caracterizado pelo **cultivo simultâneo de mais de uma espécie vegetal na mesma área agrícola**. Ao realizar o consórcio em vaso de alface associado com vinca (*Catharanthus roseus*), alho (*A. sativum*), mil-folhas (*Achillea milefolium*) ou cravo-de-defunto (*Tagetes patula*), foi observada redução na população de nematoide-das-galhas (*Meloidogyne javanica*), além de efeito positivo no crescimento da alface aumentando a massa fresca da parte aérea. Portanto, estas plantas medicinais apresentam potencial para serem utilizadas no campo. Ainda são necessários mais estudos no campo para confirmar os resultados para vinca e mil-folhas, enquanto o cultivo de **cravo-de-defunto e alho (Figura 4) já apresentaram resultados em que auxiliaram na redução da**

população de nematoide-das-galhas quando cultivados no campo em consórcio com a cultura principal.

Figura 4. Cultivo de alface (cultura principal) em consórcio com alho (cultura resistente, FR ≤ 1) para o manejo de nematoide-das-galhas no campo.

4.4 Adição de matéria orgânica

A adição de matéria orgânica pode auxiliar na proteção das plantas contra o ataque de nematoides parasitas de plantas, principalmente por **melhorar as condições físicas e químicas do solo**, melhorando **o crescimento das plantas** e favorecendo a atividade dos **organismos que competem com os nematoides parasitas de plantas ou que atuam como inimigos naturais**, isso é, qualquer organismo (vírus, fungos, bactérias e nematoides predadores) que exerce controle natural sobre o nematoide-das-galhas. A matéria orgânica também pode **liberar compostos tóxicos com ação nematicida**.

4.5 Solarização

Processo utilizado para reduzir a população de nematoides parasitas de plantas no solo ou substrato através da sua **cobertura com lonas plásticas transparentes** e exposição à **insolação direta** por **pelo menos 4 a 6 semanas**, podendo ficar por mais tempo. O

aquecimento do solo ou substrato provoca a morte de nematoides parasitas de plantas, assim como de outros patógenos de plantas, insetos e plantas invasoras. Realizar esse processo preferencialmente no verão, para obter maior insolação e calor. **Remover a lona plástica somente no momento do plantio** e evitar o revolvimento do solo ou substrato para não ocorrer inversão da camada solarizada.

Preparo do solo ou substrato para realizar a solarização:

- **Remover restos vegetais e detritos (galhos, pedras, ...) que possam perfurar ou danificar a lona plástica**, para evitar perdas de calor.
- Deixar o **solo úmido**, mas não encharcado.

4.6 Uso de cultivares de alface resistentes ao nematoide-das-galhas

Existem **cultivares de alface que são resistentes ao nematoide-das-galhas** e estão disponíveis no mercado. Na tabela abaixo estão listadas algumas destas cultivares.

Tabela 1. Cultivares de alface que são resistentes ao nematoide-das-galhas.

Cultivar	Tipo	Resistência de Meloidogyne
Calgary	Americana	*M. incognita* raça 2
Calona	Americana	*M. incognita* raças 1 e 2, *M. javanica*, *M. enterolobii*
Challenge	Americana	*M. incognita* raças 1 e 2, *M. javanica*, *M. enterolobii*
Classic	Americana	*M. incognita* raças 1 e 2, *M. javanica*, *M. enterolobii*
Coolguard	Americana	*M. incognita* raça 2
Desert Queen	Americana	*M. incognita* raças 1 e 2, *M. javanica*, *M. enterolobii*
Grand Rapids	Crespa	*M. incognita*, *M. javanica*, *M. enterolobii*
IP-11	Americana	*M. incognita* raças 1 e 2, *M. javanica*, *M. enterolobii*
Ithaca	Americana	*M. incognita* raças 1 e 2, *M. javanica*, *M. enterolobii*
Jackal	Americana	*M. incognita* raça 2
L 104	Americana	*M. incognita* raças 1 e 2, *M. javanica*, *M. enterolobii*
La Jolla	Americana	*M. incognita* raça 2
Lady	Americana	*M. incognita* raça 2, *M. javanica*
Legacy	Americana	*M. incognita*
Lorca	Americana	*M. incognita*
Raider Plus	Americana	*M. incognita* raças 1 e 2, *M. javanica*, *M. enterolobii*
Robinson	Americana	*M. incognita* raça 2, *M. javanica*
Romana Balão	Crespa	*M. incognita* raça 1, *M. javanica*
RS 1397	Americana	*M. incognita* raças 1 e 2, *M. javanica*, *M. enterolobii*
Salad Bowl Roxa	Crespa	*M. javanica*
Salinas 88	Americana	*M. incognita* raças 1 e 2, *M. javanica*, *M. enterolobii*
Vanguard 75	Americana	*M. incognita* raças 1 e 2, *M. javanica*, *M. enterolobii*
Winterset	Americana	*M. incognita* raça 2, *M. javanica*

Fonte: Colariccio e Chaves (2017).

4.7 Controle químico

O controle químico é feito a partir do uso de nematicidas químicos, todavia, devido à sua toxicidade e ao longo período em que estes permanecem no solo, o seu uso deve ser feito com cautela. **Não há registro de nematicidas químicos liberados para uso na cultura da alface**, visto que alface é uma cultura de ciclo curto e que é consumida *in natura*.

4.8 Controle biológico

O controle biológico está crescendo como estratégia de manejo de nematoides parasitas de plantas devido às preocupações ambientais e demandas da agricultura orgânica, apresentando menor impacto ao meio ambiente do que os nematicidas químicos.

Para obter resultado eficaz utilizando produtos biológicos, é **imprescindível respeitar as recomendações do fabricante** com relação à dosagem, forma e período de aplicação.

A aplicação de *Bacillus subtilis* BV09 (Biobaci®) em cultivos de alface auxiliou na redução da população de *M. incognita* no campo.

No Brasil existem atualmente diversos **produtos biológicos disponíveis no mercado para o controle de nematoides parasitas de plantas**, sendo observados 47 nematicidas microbiológicos disponíveis no AGROFIT em Janeiro de 2022 (Tabela 2). Foram observadas 11 diferentes espécies de microrganismos nestas formulações, sendo compostas em sua maioria por uma única espécie de bactéria ou de fungo, mas também foram encontradas formulações a base de misturas com mais de uma espécie de bactérias e fungos.

Tabela 2. Nematicidas microbiológicos registrados para controle de nematoide-das-galhas no Ministério da Agricultura.

Marca Comercial	Ingrediente Ativo	Alvos
AgDommon; Nemaoff;	*Bacillus licheniformis + Bacillus subtilis + Paecilomyces lilacinus*	*Pratylenchus brachyurus, Meloidogyne incognita*
AmyloTrop	*Bacillus amyloliquefaciens*	*P. brachyurus, M. incognita*
Andril Prime	*Bacillus firmus*	*P. brachyurus, M. javanica*
Arvatico	*Bacillus velezensis*	*M. incognita, Fusarium solani, Rhizoctonia solani*
Atialy; Lilatrix;	*P. lilacinus*	*M. incognita*
Aveo EZ	*B. amyloliquefaciens*	*M. incognita, P. brachyurus, Heterodera glycines*
Bio Tramo	*B. licheniformis + B. subtilis + P. lilacinus*	*P. brachyurus, M. incognita*
Biobaci; Baci-Attack; Baci-Guard;	*B. subtilis*	*Meloidogyne exigua, M. javanica, M. incognita, Meloidogyne paranaensis, Pratylenchus zeae, Fusarium oxysporum*
Bioessence Drive	*B. subtilis*	*M. javanica, M. incognita, H. glycines, P. brachyurus, R. solani, Sclerotinia sclerotiorum*
Biostat WP	*Purpureocillium lilacinum*	*M. javanica, M. incognita*

Fonte: Agrofit (2022).

Tabela 2. Nematicidas microbiológicos registrados para controle de nematoide-das-galhas no Ministério da Agricultura (continuação).

Marca Comercial	Ingrediente Ativo	Alvos
BN40.001/19*	*P. lilacinus*	*M. incognita*
Boneville	*B. amyloliquefaciens*	*M. javanica, M. incognita, P. brachyurus*
Certano	*B. velezensis*	*M. incognita, F. solani, R. solani*
Chevelle	*B. amyloliquefaciens*	*M. javanica, M. incognita, P. brachyurus*
Furatrop; Nematrop;	*B. subtilis*	*M. javanica, M. incognita, H. glycines, P. brachyurus, R. solani, S. sclerotiorum*
Inlayon Eco	*B. amyloliquefaciens*	*M. javanica, P. zeae, P. brachyurus*
Lalnix Resist	*Trichoderma endophyticum*	*M. incognita, P. brachyurus, H. glycines*
Loyalty Bio; Trunemco;	*B. amyloliquefaciens*	*M. javanica, M. incognita, P. brachyurus, Rotylenchulus reniformis*
Lumialza	*B. amyloliquefaciens*	*M. incognita, P. brachyurus, H. glycines*
Messenger	*B. licheniformis + B. subtilis + P. lilacinus*	*M. incognita, P. brachyurus*
Nemacontrol Super	*B. amyloliquefaciens*	*M. javanica, P. zeae, P. brachyurus*
Nemakill	*P. lilacinus*	*M. incognita*
Nemaster	*B. subtilis*	*M. javanica, M. incognita, H. glycines, P. brachyurus, R. solani, S. sclerotiorum*
Nemat	*P. lilacinus*	*M. javanica, M. incognita, P. brachyurus*
Nettus*	*P. lilacinus*	*M. incognita*
No-Nema; Nema-Attack; Nema-Guard;	*B. amyloliquefaciens*	*M. javanica, M. incognita, P. brachyurus, H. glycines, Fusarium verticillioides, Macrophomina phaseolina*
Oleaje Prime	*B. firmus*	*M. javanica, P. brachyurus*
Onix	*Bacillus methylotrophicus*	*M. javanica, P. brachyurus*
Onix OG*	*B. methylotrophicus*	*M. javanica, P. brachyurus*
Paladyo	*B. subtilis*	*M. javanica, M. incognita, H. glycines, P. brachyurus, R. solani, S. sclerotiorum*
Presence; Fortmax;	*B. licheniformis + B. subtilis*	*M. incognita, P. brachyurus*
Profix; Volga;	*B. licheniformis + B. subtilis + P. lilacinus*	*M. incognita, P. brachyurus*
Profix-D	*B. licheniformis + B. subtilis + P. lilacinus*	*M. incognita, P. brachyurus*
Profix-E	*B. licheniformis + B. subtilis + P. lilacinus*	*M. incognita, P. brachyurus*
Profix-F	*B. licheniformis + B. subtilis + P. lilacinus*	*M. incognita, P. brachyurus*
Profix-G	*B. licheniformis + B. subtilis + P. lilacinus*	*M. incognita, P. brachyurus*
Purpureonyd FR 25	*P. lilacinus*	*M. incognita*
Quartzo; Surface;	*B. licheniformis + B. subtilis*	*M. exigua, Meloidogyne graminicola, M. incognita, M. javanica, H. glycines, P. brachyurus, P. zeae, Radopholus similis*
Rizos	*B. subtilis*	*M. javanica, P. brachyurus*
Rizos OG*	*B. subtilis*	*M. javanica, P. brachyurus*
Rizotec; Rizotec Crops;	*Pochonia chlamydosporia*	*M. javanica*
RIZO-TURBO; PC-Attack; PC-Guard;	*P. chlamydosporia*	*M. javanica, M. incognita*
Trichodermil Evo OD	*Trichoderma harzianum*	*M. incognita, H. glycines, P. brachyurus, F. solani, R. solani, S. sclerotiorum, M. phaseolina*
Veraneio	*B. amyloliquefaciens*	*M. incognita, M. javanica, P. brachyurus*
Votivo Prime	*B. firmus*	*M. javanica, M. graminicola, H. glycines, P. brachyurus, R. reniformes*

* Produto Fitossanitário com Uso Aprovado para a Agricultura Orgânica. **Fonte:** Agrofit (2022).

Para consultar novas atualizações sobre o registro de produtos para o controle de nematoides parasitas de plantas é só acessar o site da Agrofit, que é um site do MAPA (Ministério da Agricultura, Pecuária e Abastecimento) onde é possível consultar os produtos que são liberados para serem utilizado em todas as culturas. Para acessar o site da Agrofit basta usar o link ou QR Code disponíveis abaixo:

https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons

4.9 Pousio/alqueive úmido

No pousio/alqueive o **solo é preparado e mantido livre de qualquer vegetação por um determinado período**. Trata-se de uma estratégia eficiente para reduzir a população de nematoide-das-galhas, pois **este nematoide não sobrevive no solo sem a presença de plantas hospedeiras**. Ao irrigar o solo, a **água induz a eclosão dos nematoides, os quais acabam morrendo no solo sem encontrar plantas para se alimentar e reproduzir**.

A realização do pousio irrigado durante 146 dias promoveu redução na população de *M. incognita*, porém, como a área ficou improdutiva durante muito tempo, houve prejuízo econômico. Quando o alqueive úmido é realizado após a **aração e irrigação do solo**, esta estratégia pode ser realizada durante períodos menores, em torno de 14 dias.

4.10 Evitar que o nematoide-das-galhas se espalhe pela propriedade

Por último, porém não menos importante, algumas medidas devem ser tomadas para evitar que o nematoide-das-galhas se espalhe por toda sua propriedade rural. São elas:

- **Uso de sementes e mudas sadias e isentas de nematoides parasitas de plantas**;
- **Limpeza das máquinas, implementos e ferramentas após serem utilizadas em áreas infestadas com nematoides parasitas de plantas**.

5. Monitoramento frequente

Se o nematoide-das-galhas está presente em sua propriedade, deve ser realizado o monitoramento frequente para evitar surpresas negativas em lavouras futuras. É importante acompanhar **quais são as espécies presentes** e o **nível populacional de cada uma delas**. Dessa forma, será possível verificar quais estratégias de manejo estão sendo mais eficientes e, com o tempo, você irá aprender a conviver com os nematoides parasitas de plantas reduzindo os danos às suas culturas.

6. Considerações finais

Converse sempre com o profissional que estiver lhe orientando. Busque entender o que está acontecendo na sua propriedade e o motivo de estar realizando cada estratégia de manejo, pois a **sua participação é essencial para o sucesso do manejo integrado de nematoides parasitas de plantas**.

Referências

ABD-ELGAWAD, M. M. M.; ASKARY, T. H. Factors affecting success of biological agents used in controlling the plant-parasitic nematodes. **Egyptian Journal of Biological Pest Control**, v. 30, n. 1, p. 17, 24 dez. 2020.

AGROFIT. **Sistema de Agrotóxicos Fitossanitários**. Disponível em: <https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons>. Acesso em: 20 jan. 2022.

CHARCHAR, J. M.; MOITA, A. W. **Metodologia para seleção de hortaliças com resistência a nematóides: Alface/Meloidogyne spp.** Brasília: Embrapa, 2005.

COLARICCIO, A.; CHAVES, A. L. R. **Aspectos Fitossanitários da Cultura da Alface**. São Paulo: Instituto Biológico, 2017.

GOULART, A. M. C. **Análise de Dados em Estudos de Diversidade de Nematoides**. Planaltina, DF: Embrapa Cerrados, 2009.

MACHADO, A. C. Z. Bionematicides in Brazil: an emerging and challenging market. **Revisão Anual de Patologia de Plantas**, p. 35–49, 10 jan. 2022.

MOREIRA, F. M. S. et al. **O ecossistema solo : componentes, relações ecológicas e efeitos na produção vegetal**. Lavras: Editora UFLA, 2013.

SIVASUBRAMANIAM, N.; HARIHARAN, G.; ZAKEEL, M. C. M. Sustainable Management of Plant-Parasitic Nematodes: An Overview from Conventional Practices to Modern Techniques. In: ANSARI, R. A.; RIZVI, R.; MAHMOOD, I. (Eds.). . **Management of Phytonematodes: Recent Advances and Future Challenges**. Singapore: Springer, 2020.

p. 353–399.

WILSON, M. J.; JACKSON, T. A. Progress in the commercialisation of bionematicides. **BioControl**, v. 58, n. 6, p. 715–722, 9 dez. 2013.

YEATES, G. W. et al. Feeding habits in soil nematode families and genera-an outline for soil ecologists. **Journal of nematology**, v. 25, n. 3, p. 315–31, set. 1993.