PETERSON SYLVIO DE OLIVEIRA NUNES

MICROBIAL CONSORTIUM OF BIOLOGICAL CONTROL AGENTS FOR PLANT PROTECTION AND GROWTH PROMOTION

LAVRAS-MG
2025

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Prof. Dr. Wagner Bettiol
Orientador

Dr. Gabriel Moura Mascarin
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2025

Ficha de identificação da obra elaborada pelo(a) autor(a) através do Sistema de Geração Automática de Ficha Catalográfica da Biblioteca Universitária da UFLA.

Sylvio de Oliveira Nunes, Peterson.
Microbial consortium of biological control agents for plant protection and growth promotion / Peterson Sylvio de Oliveira Nunes. 2024.
132 p.

Orientador: Wagner Bettiol
Coorientador: Gabriel Moura Mascarin
Tese (Doutorado) - Universidade Federal de Lavras, 2024.
Bibliografia.

1. Consórcios de microrganismos. 2.Trichoderma. 3. Fungos entomopatógenos. I. Bettiol, Wagner. II. Moura Mascarin, Gabriel. III. Universidade Federal de Lavras. IV. Título.

PETERSON SYLVIO DE OLIVEIRA NUNES

CONSÓRCIO MICROBIANO DE AGENTES DE CONTROLE BIOLÓGICO PARA PROTEÇÃO DE PLANTAS E PROMOÇÃO DO CRESCIMENTO

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Tese APROVADA em 23 de março de 2024.
Membros da banca:

Dr. Wagner Bettiol- Presidente – EMBRAPA MEIO AMBIENTE
Dr. Flávio Henrique Vasconcelos de Medeiros- UFLA
Dr. Gabriel Moura Mascarin- EMBRAPA MEIO AMBIENTE
Dra. Laura Bononi- NOOA
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LAVRAS-MG
2025

AGRADECIMENTOS

À Universidade Federal de Lavras e ao Departamento de Fitopatologia e todos seus funcionários, pela oportunidade e aprendizado.

Ao Prof. Dr. Wagner Bettiol, pela orientação, apoio, confiança, paciência, ajuda, dedicação, incentivo e pelos conhecimentos transmitidos.

Ao Dr. Gabriel Moura Mascarin, pela coorientação, suporte e ensinamentos.

Ao CNPq, pela concessão de bolsa de estudos e recursos para execução do projeto.

À Empresa Brasileira de Pesquisa Agropecuária – Embrapa Meio Ambiente, pela oportunidade e apoio concedidos para meu aperfeiçoamento pessoal e profissional.

Aos meus familiares, em especial à minha mãe e as minhas irmãs, por todo apoio, confiança e incentivo. Ao Eliton pela parceria e apoio.

À secretária da pós-graduação Ariane, pelos aconselhamentos, amizade e por toda ajuda e colaboração.

Aos funcionários da Embrapa Meio Ambiente: Neusa Domingos, Rosely Nascimento, Ana Carolina e Antônio Souza, pela colaboração e amizade.

Aos amigos da UFLA, do departamento de Fitopatologia e da Embrapa Meio Ambiente, que tornaram essa caminhada mais fácil.

Enfim, agradeço a todos que tornaram esse sonho possível.

MUITO OBRIGADO!

RESUMO

Os micro-organismos benéficos desempenham um papel crucial na agricultura moderna, atuando como biopesticidas, biostimulantes/biofertilizantes e mitigadores de estresse abiótico em culturas. Suas funções multifacetadas contribuem significativamente para a saúde e a sustentabilidade das colheitas, alinhando-se com os princípios da agricultura regenerativa ao minimizar as pegadas de carbono e reduzir a dependência de insumos agrícolas químicos. O conceito de 'consórcios microbianos', que envolve a combinação de múltiplas espécies ou cepas de fungos e bactérias, tem recebido reconhecimento por seus potenciais benefícios em comparação com as aplicações de espécies/cepas únicas. Este método visa empregar um espectro de funções ecológicas, incentivando o equilíbrio biológico dentro dos sistemas agrícolas. Pesquisas recentes destacam como os consórcios microbianos podem melhorar diretamente e indiretamente a saúde das plantas, por meio de diversos mecanismos e interações com suas plantas hospedeiras. Esta revisão apresenta exemplos que demonstram a eficácia dos consórcios microbianos como biopesticidas e biostimulantes/biofertilizantes. Esses consórcios mostram potencial no manejo de doenças e pragas em plantas, ao mesmo tempo em que promovem o crescimento das plantas e mitigam estresses abióticos específicos em culturas. Além disso, esta tese explora, no capítulo um, uma revisão sobre o uso recente e as perspectivas futuras de consórcios microbianos na agricultura, apresentando exemplos do dinâmico mercado brasileiro de produtos biológicos. No capítulo dois, são explorados o duplo potencial de fungos, tanto para controle de pragas como para controle de doenças de plantas, podendo ser utilizados no manejo integrado. Esta tese visa não apenas destacar as possibilidades emergentes nesse campo, mas também fornecer exemplos sobre estratégias promissoras para manejo da murcha do feijoeiro e mosca branca.

Palavras-chave: Consórcios microbianos, Fungos entomopatogênicos, *Trichoderma*, *Beauveria*

ABSTRACT

Beneficial microorganisms play a crucial role in modern agriculture, serving as biopesticides, biostimulants/biofertilizers, and abiotic stress mitigators in crops. Their multifaceted functions significantly contribute to crop health and sustainability, aligning with the principles of regenerative agriculture by minimizing carbon footprints and reducing reliance on chemical agricultural inputs. The concept of 'microbial consortia,' involving the combination of multiple species or strains of fungi and bacteria, has gained recognition for its potential benefits compared to single-species/strain applications. This method aims to employ a spectrum of ecological functions, encouraging biological balance within agricultural systems. Recent research highlights how microbial consortia can directly and indirectly improve plant health through various mechanisms and interactions with their host plants. This review presents examples demonstrating the efficacy of microbial consortia as biopesticides and biostimulants/biofertilizers. These consortia show potential in managing plant diseases and pests while promoting plant growth and mitigating specific abiotic stresses in crops. Additionally, this thesis explores, in Chapter One, a review of the recent use and future prospects of microbial consortia in agriculture, presenting examples from the dynamic Brazilian market of biological products. In Chapter Two, the dual potential of fungi is explored, both for pest control and for plant disease control, which can be utilized in integrated pest management. This thesis aims not only to highlight emerging possibilities in this field but also to provide examples of promising strategies for managing bean wilt and whitefly infestations.

Keywords: Microbial consortia, Entomopathogenic fungi, *Trichoderma, Beauveria*

INDICADORES DE IMPACTO

Impactos sociais, tecnológicos, econômicos e culturais

O uso de microrganismos benéficos na agricultura oferece varios benefícios sociais. Em primeiro lugar, a redução do uso de insumos agrícolas químicos, como pesticidas e fertilizantes sintéticos, tem impactos diretos na saúde das comunidades rurais, que frequentemente estão expostas a esses produtos. A redução dos resíduos químicos no ambiente também reduz os riscos para os consumidores e para os ecossistemas. Além disso, a adoção de tecnologias como os consórcios microbianos pode contribuir para a inclusão social, ao fornecer aos pequenos produtores e agricultores familiares acesso a soluções agrícolas inovadoras e de baixo custo, promovendo a justiça social. O impacto econômico e tecnológico dos consórcios microbianos na agricultura é imenso. Economicamente, essas tecnologias podem reduzir custos, aumentar a produtividade e criar novos mercados, contribuindo para a sustentabilidade financeira dos agricultores. No aspecto tecnológico, os avanços na biotecnologia e em tecnologias digitais oferecem soluções cada vez mais eficazes e precisas para os desafios enfrentados no campo. Juntas, essas mudanças estão moldando uma nova era na agricultura, onde a inovação, a sustentabilidade e a eficiência caminham lado a lado, criando um futuro mais promissor tanto para os produtore quanto para os consumidores. Além disso, a adoção dessas tecnologias tem caráter extensionista, sendo amplamente utilizada por produtores em diversas regiões, o que facilita a difusão do conhecimento e a implementação de práticas sustentáveis em áreas rurais.

IMPACT INDICATORS

Social, technological, economic and cultural impacts

The use of beneficial microorganisms in agriculture offers various social benefits. First, the reduction in the use of chemical agricultural inputs, such as pesticides and synthetic fertilizers, has direct impacts on the health of rural communities, which are often exposed to these products. The reduction of chemical waste in the environment also lowers the risks for consumers and ecosystems. Additionally, the adoption of technologies such as microbial consortia can contribute to social inclusion by providing small producers and family farmers with access to innovative, low-cost agricultural solutions, promoting social justice. The economic and technological impact of microbial consortia in agriculture is immense. Economically, these technologies can reduce costs, increase productivity, and create new markets, contributing to the financial sustainability of farmers. From a technological standpoint, advancements in biotechnology and digital technologies offer increasingly effective and precise solutions to the challenges faced in the field. Together, these changes are shaping a new era in agriculture, where innovation, sustainability, and efficiency go hand in hand, creating a more promising future for both producers and consumers. Furthermore, the adoption of these technologies has an extensionist character, being widely used by producers in various regions, which facilitates the spread of knowledge and the implementation of sustainable practices in rural areas.

SUMÁRIO

1. INTRODUÇÃO GERAL

O comportamento dos consumidores vem mudando a agricultura tradicional. A preocupação com a saúde, segurança alimentar e com o impacto ambiental causado pelo uso indiscriminado de inseticidas, fungicidas e fertilizantes inorgânicos são os principais responsáveis por essa mudança. Deste modo, uma prática que vem crescendo na agricultura é o uso de produtos menos agressivos, dentre eles os biofungicidas, biopesticidas e bioestimulantes. Diversos estudos com microrganismos agentes de controle biológico de pragas e doenças e como biofertilizantes estão sendo desenvolvidos, compreendendo o isolamento, a bioprospecção, a seleção, a fermentação e a formulação desses microrganismos (Bononi et al., 2020; Pacifico et al., 2021; De Sá Santos et al., 2021; Marciano et al., 2021; Singh et al., 2021; Fontana, et al., 2021; Mascarin et al., 2022).

O mercado de biopesticidas também vem mostrando essa mudança na agricultura. O valor de mercado de biopesticidas foi estimado em US$ 14,6 bilhões de dólares em 2023, devendo atingir US$ 27,9 bilhões de dólares em 2028, com taxa de crescimento de 13,8% (Markets; Markets, 2020). No Brasil, o mercado de biodefencivos cresceu 62% de 2018 a 2022. Com um crescimento de 219% entre a safra 2019/2020 e a 2021/202. Estima-se que o mercado de bioprodutos atinja 3, bilhões de dólares em 2030, representando um crescimento de 107% em relação ao ano de 2021 (Croplife Brasil, 2024). Atualmente, existem registrados no Brasil 526 produtos microbiológicos, desses 290 inseticidas, 109 biofungicidas, 69 bionematicidas, 53 acaricidas e cinco bactericidas (Agrofit, 2024).

Um expressivo aumento no número de produtos registrados para o controle biológico foi observado no Brasil nos últimos anos (Croplife Brasil, 2024). Contudo, apesar do expressivo crescimento do setor de biodefensivos, os métodos de produção e processos empregados na indústria não evoluíram no ritmo necessário, havendo grandes gargalos a serem superados, como a baixa vida-de-prateleira (Faria; Wraight, 2021). Entretanto, mesmo com o acentuado crescimento do mercado de biopesticidas, as dificuldades enfrentadas na multiplicação, armazenamento, comercialização e, principalmente, no desenvolvimento de formulações estáveis e com vida de prateleira adequada, afetam seriamente a oferta e a qualidade de produtos no mercado. Como consequência, o desenvolvimento de novas formulações que possam levar ao aprimoramento de agentes ativos constitui um importante desafio econômico e tecnológico (Faria et al., 2010).

Além disso, o mercado de produtos biológicos está passando por uma transformação marcante, caracterizada pelo aumento da utilização de produtos com consórcios de microrganismos. Essa tendência torna-se evidente ao observarmos o aumento significativo no registro de produtos que contêm mais de um microrganismo em sua composição, conforme destacado por Nunes et al. (2024). Além disso, a literatura científica apresenta inúmeros estudos que corroboram a perspectiva promissora dos consórcios de microrganismos (Anshu et al., 2022; Bo et al., 2022; Bouchard-Rochette et al., 2022; De Vrieze et al., 2018; Devi et al., 2022; Fukui et al., 1999; Karthiba et al., 2010; Kaur et al., 2022; Kim et al., 2008; Krzyzanowska et al., 2019; Kumawat et al., 2022; Metwally et al., 2022; Monteiro et al., 2013; Muthukumar et al., 2011; Muthuraja and Muthukumar, 2022; Nafady et al., 2022; Slininger et al., 2007; Soth et al., 2022; Srinivasan and Mathivanan, 2009; Stummer et al., 2022). O Mercado brasileiro a adoção e registro de produtos com múltiplos gêneros, espécies e/ou cepas já é uma realidade. Na totalidade, 63 produtos registrados no Ministério da Agricultura, Pecuária e Abastecimento (MAPA) contêm mais de um microrganismo. Os principais produtos contendo consórcio estão divididos em três categorias, sendo elas: inseticida microbiológico, fungicida microbiológico e nematicida microbiológico (AGROFIT, 2024).

Mas o que é o consorcio de microrganismos? Consórcio microbiano é, geralmente, referido como um grupo de diversos microrganismos que têm a capacidade de viver em conjunto em uma comunidade (Ram et al., 2022). Esse consórcio pode ser construído artificialmente por meio da mistura de microrganismos que não ocupam o mesmo nicho ecológico (Arif et al., 2020). Esta tecnologia fundamenta-se na sinergia entre os microrganismos consorciados que são capazes de interagir e aprimorar seus efeitos, tanto na mitigação da severidade de doenças quanto na atuação em múltiplos alvos biológicos, caracterizando-a como uma abordagem de múltiplos sítios biológicos. Diferentes microrganismos são investigados em consórcio, destacando-se representantes dos gêneros *Trichoderma* (Stummer et al., 2022), *Bacillus* (Bouchard-Rochette et al., 2022), *Metarhizium* (Upamanya et al., 2020), *Beauveria* (Soth et al., 2022) e *Pseudomonas* (Kaur et al., 2022). Os benefícios proporcionados pelos consórcios microbianos na agricultura abrangem o controle de fitopatógenos (Zitlalpopoca-Hernandez et al., 2022; Nafady et al., 2022; Bouchard-Rochette et al.,2022), o manejo de pragas (Spescha et al., 2023; Metwally et al., 2022; Soth et al., 2022), mitigação do estresse biótico ou abiótico e/ou o estímulo ao crescimento vegetal (Dixit et al., 2023; Li et al.,2022).

Microrganismos presentes em consórcio têm a capacidade de desempenhar diversas funções que seriam impraticáveis para um único organismo. Esse consórcio favorece a sinergia entre diferentes modos de ação, tais como micoparasitismo, patogenicidade a pragas, competição, antibiose, resistência sistêmica induzida, produção de hormônios, solubilização de fosfatos, fixação de nutrientes, resistência a raios UV, entre outros.

A aplicação de consórcios também pode impactar positivamente a estrutura biológica do solo, estimulando o crescimento de microrganismos benéficos e melhorando a fertilidade do solo, conforme indicado por estudos recentes (Shukla et al., 2022). Essa aplicação conjunta de microrganismos resulta em aumento do carbono na biomassa microbiana, total disponibilidade de nitrogênio, potássio e fósforo, além de restaurar a diversidade microbiana na rizosfera e modular a atividade enzimática do solo. Além disso, microrganismos desempenham papel crucial na decomposição de resíduos orgânicos, incluindo lignina e celulose, contribuindo para o armazenamento de carbono no solo. A biomassa microbiana do solo, composta por organismos com volume inferior a cerca de 5×10^3 μm^3, desempenha um papel vital na conversão de resíduos orgânicos em biomassa ou na mineralização em CO_2 e H_2O. Entretanto, algumas dificuldades são encontradas na confecção de um consorcio de microrganismo. O principal obstáculo a confecção de um consorcio e avaliar as possíveis interações que podem ocorrer entre os microrganismos, poucas metodologias estão disponíveis na literatura e nem sempre são eficientes na avaliação da compatibilidade (Nunes et al., 2024).

Os consórcios podem gerar efeito sinérgico, antagônico e neutro (Xu et al., 2011a). Uma escolha inadequada pode resultar em um consórcio ineficaz, no qual uma cepa exerce impacto negativo sobre a outra, seja através da diminuição do crescimento, competição ou até mesmo pela emissão de substâncias inibitórias. (Felici et al., 2008; Roberts et al., 2005; Sarma et al., 2015; Stockwell et al., 2010a). Na planta, um consorcio antagônico, incompatível, pode proporcionar uma menor eficiência para o controle de patógenos ou para a promoção de crescimento vegetal (Cruz-Magalhães et al., 2022). Em outras palavras, é fundamental examinar potenciais incompatibilidades entre os microrganismos que serão co-produzidos ou co-formulados, a fim de desenvolver produtos consorciados (Minchev et al., 2021). Vale ressaltar, que as cepas selecionadas devem ser capazes de realizar sua função individualmente e quando aplicadas juntas no consórcio, sua atividade deve ser mantida ou melhorada.

Em síntese, o surgimento dos consórcios de microrganismos marca uma importante etapa do controle biológico. Destacando-se como uma estratégia promissora na agricultura, capaz de proporcionar benefícios abrangentes, desde o controle de patógenos até o estímulo ao

crescimento vegetal. Contudo, é crucial enfrentar os desafios associados à avaliação da compatibilidade entre os microrganismos nos consórcios, garantindo a eficácia na aplicação. À medida que o mercado de produtos biológicos continua a se transformar, a adoção e o aprimoramento contínuo das tecnologias emergentes são essenciais para uma agricultura mais sustentável e eficiente no futuro.

No presente trabalho, são explorados dois capítulos fundamentais. No primeiro, abordamos uma revisão abrangente do mercado de produtos biológicos que incluem misturas de microrganismos, apresentamos os principais métodos para avaliar a compatibilidade entre esses microrganismos consorciados. Neste capítulo, buscamos fornecer uma visão panorâmica dos consórcios presentes no mercado brasileiro. No capítulo dois, apresentamos a elaboração de um consórcio com eficiência de controle tanto de doenças como de pragas, destacando a versatilidade e o duplo potencial de controle dos microrganismos avaliados. Demonstramos também a efetividade do controle, tanto *in vivo* quanto *in vitro,* de um fungo entomopatogênico no combate ao Fusarium no feijoeiro. Além disso, enfatizamos a eficácia do Trichoderma no controle da mosca branca. A mosca branca é uma das principais pragas da cultura do feijão e, além dos danos diretos que ela causa, pode ser um vetor de viroses.

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Artigo 1: MICROBIAL CONSORTIUM OF BIOLOGICAL PRODUCTS: DO THEY HAVE A FUTURE?

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Received Date:3 September 2023
Revised Date:1 January 2024
Accepted Date:2 January 2024

DOI: https://doi.org/10.1016/j.biocontrol.2024.105439

ABSTRACT

Beneficial microbes play crucial role in modern agriculture, serving as biopesticides, biostimulants/biofertilizers, and mitigating abiotic stress in crops. Their multifaceted functions contribute significantly to crop health and sustainability, aligning with the principles of regenerative agriculture by minimizing carbon footprints and reducing dependence on agrichemical inputs. The concept of 'microbial consortia', involving the combination of multiple fungal and bacterial species or strains, has gained recognition for its potential advantages over single species/strains applications. This method aims to employ a spectrum of ecological functions, encouraging biological equilibrium within agricultural systems. Recent research highlights how microbial consortia can directly and indirectly enhance plant health, through various mechanisms and interactions with their host plants. This review presents examples demonstrating the efficacy of microbial consortia as biopesticides and biostimulants/biofertilizers. These consortia exhibit potential in managing plant diseases and pests, while also promoting plant growth and mitigating specific abiotic and biotic stresses in crops. This review examines real-world examples considering instances of success and failure, discusses methodologies employed for evaluating interactions, and also addresses challenges in the selection, production, and application of optimal microbial consortia for agricultural use providing valuable insights in the current and future prospects of microbial consortia in modern agriculture.

Keywords: sustainable agriculture, biological control, plant protection, microbial revolution, synthetic microbiomes, bioinputs.

1.Introduction

Microbial consortia comprise two or more microbial species living together (Ram et al., 2022). In nature, microbial organisms associated with plants or animals rarely interact with their hosts in isolation; rather, their impacts reflect co-evolved interactions with other microbes. These consortia act in biotic and abiotic interactions with plants often within symbiotic interactions, and their co-adaption may perform important agroecological functions (Bhatt et al., 2021).

Recently, there has been interest in combining microbial species that do not naturally occupy the same ecological niche (Arif et al., 2020). Such artificial microbial consortia were initially developed for the pharmaceutical and food industries (Ergal et al., 2022). Probiotics, for example, have received recent attention for their beneficial roles in human and animal health, such as modulating gut microbiota and immune function (Wang et al., 2021).

In agriculture, the use of multiple microorganisms have received attention due to their capacity to provide ecological functions, including soil bioremediation (Rajpal et al., 2022; Siripattanakul-Ratpukdi et al., 2014), plant growth promotion (Chen et al., 2021; Kaur et al., 2022; Muthuraja and Muthukumar, 2022) and pest and disease suppression (Muthuraja and Muthukumar, 2022; Nafady et al., 2022; Nawaz et al., 2020; Soth et al., 2022; Stummer et al., 2022; Upamanya et al., 2020).

Benefits of microbial consortia include overcoming the biological limitations associated with single microorganisms. For example, the combination of multiple microbes is more stable across a wider variety of ecological and environmental conditions (Qian et al., 2020; Tabacchioni et al., 2021; Trivedi et al., 2021). Combining microorganisms with different modes of action, nutritional requirements and growth characteristics is desirable, especially if synergistic interactions occur (Ram et al., 2022). Additional advantages of applying microbial consortia include reduced application costs and increase microbial biodiversity in the plant rhizosphere and phyllosphere. However, successfully implementing microbial consortia faces several challenges, including incompatibility between microorganisms, competition from native organisms, and adaptation to the habitat (Arif et al., 2020; Stockwell et al., 2010; Xu and Yu, 2021).

This review discusses recent use and future perspectives for microbial consortia in sustainable agriculture. In particular, we provide recent examples from the large Brazilian market for biological products, which increasingly containing mixtures of microbial species/strains approved for use by the Ministry of Agriculture.

2.Microbial Consortia

Natural consortia of bacteria and fungi are commonly found in all environments, including soils and plants (Ayala-Campos et al., 2022; Faria et al., 2020; Plett and Martin, 2018; Wagg et al., 2014; Zhou et al., 2022), aquatic ecosystems (Manirakiza et al., 2022), mammal intestines (Turroni et al., 2022; Yang et al., 2022) and other places (Liu and Xu, 2022). These microbial communities play important roles in ecological systems, including the organic carbon and nitrogen cycles (Massot et al., 2022) as well as human and animal health (Klimasmith and Kent, 2022; Kumar et al., 2022; Moitinho et al., 2020; Salwan and Sharma, 2022; Verma et al., 2022). Natural microbial consortia were used by ancient civilizations in the fermentation of food and beverages (Padmaperuma et al., 2020).

Interactions within microbial communities can be broadly classified as cooperative or non-cooperative (Zapalski, 2011). Cooperative (positive) microbial interactions include symbiosis, mutualism, and commensalism (Bhatia et al., 2018), whereas non-cooperative (negative) interactions include parasitism, amensalism, and predation (Jagmann and Philipp, 2014).

Microbial consortia can characterized as artificial, synthetic, or semi-synthetic (Bernstein and Carlson, 2012). According to Massot et al. (2022), artificial consortia consist of selected microbial strains that do not naturally coexist but are selected for specific functions. Synthetic consortia comprise two or more genetically modified microorganisms, while semi-synthetic consortia combine microorganisms from natural and genetically modified populations (Bernstein and Carlson, 2012). Through this approach, it is possible to build and manage consortia that can be strategically applied for agricultural applications (Padmaperuma et al., 2020). Both a priori knowledge and empirical testing is needed to develop useful artificial microbial consortia (Gehlot et al., 2021; Ghosh et al., 2016).

3.Designing a consortium of microorganisms

The selection of microorganisms involves several steps, spanning the selection of strains with the desired function, evaluating compatibility and synergism between the strains, and

performing evaluation under field conditions (Behera et al., 2021; Roell et al., 2019; Sarma et al., 2015).

Köhl et al. (2011) suggested nine screening steps: #1: Assessment of targeted crop, disease and markets; #2: Origin and isolation of candidate antagonist; #3: Preliminary assessments in rapid-throughput screening systems; #4: Identification of candidate antagonists and database mining; #5: Efficacy testing against pathogens on plants; #6: Preliminary tests on mass production; #7: Development and testing of a pilot formulation and estimation of registration costs; #8: Field testing and upscaling mass production; and #9: Integration into cropping systems. In a later paper, Bettiol et al. (2021) propose six steps, i.e., #1: Efficacy testing of pathogen control in plants; #2: Preliminary assessment of mass production potential; #3: Pilot formulation; #4: Upscaling of mass production; #5: Full field testing; and #6: Integration into crop systems management. These authors recommend the same strains should be checked multiple times to ensure consistency in performance.

In practice, however, few studies developing microbial consortia follow these guidelines. For example, few studies provide a detailed selection criterion for microorganisms comprising the consortium (Bouchard-Rochette et al., 2022; Cruz et al., 2006; Jain et al., 2012; Karthiba et al., 2010; Stuart et al., 2020; Kaur et al., 2022; Krzyzanowska et al., 2019; Kumawat et al., 2022; Slininger et al., 2007; Soth et al., 2022; Stummer et al., 2022; Xu et al., 2011). Rather, most authors only consider the effects of individual microbial species/strains, without exploring potential intra specific interactions (Shang and Liu, 2021). Although evaluating efficacy is important, relatively few studies quantify the effectiveness of consortia in comparison to the efficiency of the strains applied singly (Xu et al., 2011). Consequently, the synergistic benefits of using a microbial consortium, including interactions with the native microbes, and the environmental, are difficult to quantify.

4.Methods to assess compatibility between microbial strains/species *in vitro*

Understanding potential interactions between microorganisms is essential, since using compatible strains, including those with synergistic effects, brings numerous potential advantages (Tables 1, 2 and 3). Studies have demonstrated that competition between plant pathogenic fungi and bacteria cultivated in the same liquid culture medium can change the production of secondary metabolites and enhance the biocontrol performance (Wu et al., 2018). Conversely, incompatible interactions between microbes can lead to negative effects on plant

growth and reduced control of disease and pests (Felici et al., 2008; Roberts et al., 2005; Sarma et al., 2015; Stockwell et al., 2010a).

In general, the investigation of specific microbial interrelationships is crucial for establishing a functionally successful consortium (Minchev et al., 2021; Santiago et al., 2017; Santoyo et al., 2021; Stockwell et al., 2010). Studies to evaluate the compatibility between fungi and bacteria include *in vitro* 'dual-culture' protocols to check for antagonistic activity (Christopher et al., 2021; Elgharably and Nafady, 2021; Mohammad et al., 2015; Suryaminarsih and Surtiningsih, 2015; Upamanya et al., 2020). In this method, two strains are co-cultured in Petri dishes containing culture medium to detect inhibition zones around the growth intersection region (Box 1). Potential interactions include: compatible mixture, partially compatible mix, invasion or replacement, inhibition at the contact point, and distant inhibition due to antibiosis (Mohammad et al., 2011). Figure 1 shows examples of incompatibility with dual culture methodology.

Box 1. The compatibility *in vitro* assay (after Mohammad et al., 2011).

In the 'dual-culture' protocols, two different fungal isolates are cultured simultaneously, 4.0 cm apart, to examine possible interactions. Tests are performed in Petri dishes containing a culture medium allowing the growth of both microorganisms. Controls consist of the individual cultivation of each strain, since the growth perimeter is used for comparative evaluation of the interaction zones in paired cultures. If there is disparity in growth rates, the inoculation is performed in two stages. First, the fungus with the slowest growth is inoculated, and after two or three days of incubation, the second species is inoculated. Cultures are generally incubated at 25 ± 2 °C, and the perimeter of growth observed daily. Growth interactions are visually assessed according to established protocols.

Additional methods are described to evaluate compatibility between bacteria (Molina-Romero et al., 2017; Muñoz-Rojas et al., 2005). Molina-Romero et al. (2017) propose two bioassays for antagonism between bacterial strains by assessing antimicrobial compounds. The first method is the double-layer agar plate method, and the second the simultaneous inhibition method. In the former, only the secreted substances interact with the target microorganisms, while in the latter, both strains are cultivated together, allowing for competitive exclusion (Box 2). These methods were employed by Muñoz-Rojas et al. (2005) to verify antagonistic activity among endophytic bacteria. The results reveal potential antagonistic interactions between 55 *Gluconacetobacter* spp. strains.

Box 2: Antagonism assays to identify bacterial strains producing antimicrobial compounds (**after** Molina-Romero et al., 2017**)**

***Double agar layer method*.** Bacterial strains containing potential inhibitory substances are grown in liquid medium until the stationary phase. For this, cultures are incubated for 24 h in PY-Ca liquid medium (peptone 5 g, yeast extract 3 g, calcium chloride 0.7 g) at 200 rpm and 30 °C. Aliquots of 200 µl from bacterial suspensions are dispensed in 96 well microplates with PY-Ca using a multipoint replicator, and incubated for 48 h at 30 °C. After incubation, producer colonies are removed with a sterile glass slide and remaining bacteria are killed by exposure to chloroform vapor for 1 h. The plates are left open in a laminar flow cabinet for evaporation of residual chloroform. Simultaneously, each potential sensitive strain is incubated under the same conditions as the potential producer strains. The culture of sensitive strain is centrifuged and the cell pellet washed with sterile distilled water and then resuspended in the same volume of distilled water. The bacterial number of this suspension should contain around 5 x 10^8 CFU mL^{-1}. Bacterial suspensions are diluted (1:10) using a solution of 0.9% w/v NaCl and 15 mL is poured on solid medium PY-Ca where the producer strains were previously grown. The excess suspension on the plates is decanted into a glass beaker after 15 minutes. Plates are incubated for 48 h for visual observation of inhibition halos indicating antibacterial activity. This method demonstrated the direct interaction of sensitive and antagonistic strains. The halos indicate inhibitory activity of substances produced by a strain that diffuses in the culture medium after cell removal.

Simultaneous inhibition method. The potential sensitive strain is grown in liquid medium PY-Ca for 24 h. Twenty microliters of broth culture (containing around 5 × 10^7 CFU mL^{-1}) are streaked on solid agar PY-Ca plates and 20 µL of cell suspension (5 × 10^8 CFU mL^{-1}) of the potential producer strain are placed at the centre of the streaked plates. After 48 h of incubation at 30 °C, the presence of inhibition halos is used to quantify antibacterial effects.

4.1. Limitations of *in vitro* methods

Despite their utility, the *in vitro* tests described above cannot capture all possible interactions after application in the field (Stewart, 2001). Cruz-Magalhães et al. (2022) demonstrated incompatibility between two *Bacillus* strains (BMH and INV) through *in vitro* tests. However, the same strains still significantly reduced the number of galls and eggs of *Meloidogyne incognita* in tomato plants (Barbosa et al., 2018). In some cases, laboratory assays may fall short in identifying potential interactions between microbes and other biological control agents. For example, Monteiro et al. (2013) evaluated the entomopathogenic nematodes

Heterorhabditis bacteriophora HP88 and *Heterorhabditis indica* LPP1 with the entomopathogenic fungi *Metarhizium anisopliae* IBCB 116 and *Beauveria bassiana* ESALQ 986 to control *Rhipicephalus microplus* (Acari: Ixodidae). Despite the combination being effective (>90% control) under laboratory conditions, it was not possible to verify interactions due to the high efficacy obtained by the nematodes alone. The authors concluded that investigation under field conditions was necessary to characterize these interactions.

Another issue of *in vitro* testing concerns the limited nutrient profiles of most media. As noted by Knudsen et al. (1997), artificial media may not reflect the nutritional conditions in the target environment. Consequently, it can be difficult to draw conclusions about prospective *in vivo* interactions from *in vitro* assays. The niche overlap index (NOI), calculated as the ratio between the number of nutrient sources used by both the biocontrol agent and pathogen, has been used to infer potential competition and success of microbial consortia (Cavaglieri et al., 2004). A microbial consortium may be selected using strains that can be grown on the same nutritional source as the pathogen (Wallis, 2021). This approach can also be used to reduce intra-specific competition between microorganisms (Janisiewicz, 1996).

For the obligate symbionts that unable to be cultivated in synthetic media, such as arbuscular mycorrhizal fungi (AMF), *in vitro* assessment shows limitations (Dey and Ghosh, 2022). The mycorrhizal symbiotic relationship of AMF enhances nutrient acquisition (Garg and Singh, 2018; Lehmann and Rillig, 2015), water absorption (Augé et al., 2015), and assists host plants to cope up with abiotic stresses (Begum et al., 2022; Hashem et al., 2018). Furthermore, these fungi are able to stimulate plant growth by producing phytohormones (Hashem et al., 2018) and induce systemic protection against a wide range of pathogens through the production of antifungal compounds and competition (Kaur et al., 2022; Maurya et al., 2018; Nafady et al., 2022; Sanmartín et al., 2020; Song et al., 2013). In such cases, compatibility and efficacy tests should be performed in pots or the field (Devi et al., 2022; Elgharably and Nafady, 2021; Kaur et al., 2022; Nafady et al., 2022; Zitlalpopoca-Hernandez et al., 2022). Elgharably and Nafady (2021) showed the compatibility of several AMF including *Funneliformis geosporum*, *F. mosseae*, *Rhizophagus clarus*, and *Scutellospora persica*, with non-pathogenic *Penicillium funiculosum* and *Fusarium oxysporum* to alleviate the deleterious effects of NaCl on wheat growth. The combined inoculation decreased Na and Cl uptake by the host plant, while increasing chlorophyll, N and P contents, and K/Na ratio leading to enhanced root colonization by AMF.

4.2.Quantitative PCR (qPCR)

Although microbial consortia are being explored for pest management and improved crop performance, detailed information of their effectiveness and establishment under field conditions is still scarce. To better understand the interactions between microbes within a consortium and environmental impact, it is necessary to track the environmental fate of specific microbial strains (Romano et al., 2020). The use of real-time RT-qPCR is increasingly used for this purpose, due to its sensitivity, speed and cost-effectiveness (Hernández et al., 2020). This technique involves designing species-specific probes to amplify and detect specific DNA or RNA sequences providing a measure of the distribution of specific microbes from a consortium (Box 3). Accuracy depends on several factors, including the quality of DNA extraction, the specificity of the primer, and the sensitivity of the standard curve. qPCR results should be compared to other methods, including culture-based techniques. The insights into plant-microbiome interactions provided by next-generation sequencing (NGS) and omics-based approaches can be used to design stable and efficient microbial consortia capable of stimulating plant growth, enhancing nutrient assimilation, and pathogen suppression (Khan, 2022).

Box 3: Real-time quantitative PCR (qRT-PCR)

The design of strain-specific qPCR assays is an important tool for tracking microbial strains in agricultural production systems. Firstly, total DNA from samples is isolated using commercial kits, according to the manufacturer's instructions. These ‘biocontrol’ strains are monitored via molecular probes that target specific DNA sequences unique to a particular microbe. The design of strain-specific qPCR assays typically involves the following steps:

1. **Identification of strain-specific DNA sequences**: The first step is to identify DNA sequences that are unique to the biocontrol strain of interest.
2. **Primer design**: Primers used specifically target regions of the genome in the target strain target and no other strains in the consortium.
3. **Optimization of qPCR conditions**: The qPCR assay is optimized to ensure accurate detection of the target DNA sequence. This includes cycling conditions (denaturation, annealing, extension, and the number of cycles), MgCl2 concentration, and primer and probe concentrations.
4. **Validation of the qPCR assay**: Test validation requires testing against a range of biocontrol strains and non-target strains. A standard curve generated using a range of

target DNA concentrations and controls are used to estimate microbial numbers in samples.

Several examples confirm that qPCR can be used to assess the compatibility and establisment of specific bacterial strains with biological control potential. In studies with plant growth-promoting rhizobacteria, Samain et al. (2022) selected a consortia of *Paenibacillus* spp. and *Arthrobacter* spp. which stimulated wheat growth, as well as providing resistance against *Mycosphaerella graminicola*, and tolerance to drought stress. By using 16S rDNA-specific primers, there was an increase in root colonization by *Paenibacillus* sp. strain PB2 was enhanced, when compared with individual inoculations. Stets et al. (2015) also used strain-specific primers to confirm the establishment of the bacterium *Azospirillum brasilense* FP2 in wheat roots co-inoculated with other microbial strains. Fernandes et al. (2014) used strain-specific primers to quantify the endophytic colonization of *Gluconacetobacter diazotrophicus* in sugarcane plants. In these cases, a cultivation-dependent approach was unsuccessful.

5.Axenic culture *versus* microbial consortia

Traditionally, most microbial strains used as biological control agents are produced and formulated through axenic culture (Bononi et al., 2020; Conte et al., 2022; Amaral et al., 2022; Pacifico et al., 2021; Karabörklü et al., 2022; Mubeen et al., 2022; Yadav and Chandra, 2014). This approach is reflected in the global portfolio of microbial pesticides comprising single strains (AGROFIT - Sistema de Agrotóxicos Fitossanitário, 2023; Bettiol et al., 2019; Woo et al., 2014).

However, as previously noted, the action of natural microbial communities can provide advantages over axenic cultures (Behera et al., 2021; Bouchard-Rochette et al., 2022; Devi et al., 2022; Stuart et al., 2020; Kaur et al., 2022; Krzyzanowska et al., 2019; Kumawat et al., 2022; Soth et al., 2022; Stummer et al., 2022; Xu et al., 2022). It is proposed that single-strain inoculations are more sensitive to competition from indigenous microbes and environmental stresses (Trivedi et al., 2020). Furthermore, single strain inoculations typically have only one or two modes of action, limiting their effectiveness for multifunctional roles (Bhatt et al., 2021). By contrast, consortia comprising compatible microbes have more diverse modes of action (i.e., mycoparasitism, competition for nutrients, antibiosis, induction of plant resistance, phosphate solubilization and nitrogen fixation, and protection against abiotic stresses) and consequently

functional plasticity and wider adaptability to a variety of environments (Bhatia et al., 2018; Xu and Yu, 2021).

6.Bioremediation

Initially, microbial consortia were used in the production of chemical, pharmaceutical, and food products, as well as in bioremediation efforts (Morales-García et al., 2019). The use of specific microorganisms to degrade, detoxify, mineralize, or transform pollutants into non-toxic compounds, has been used to mitigate or minimize negative impacts to the environment (Azubuike et al., 2016; Singh, 2014). However, in many cases, the removal of a toxic compound can only be performed using a consortium of microorganisms with complementary functions. Individual microorganisms cannot generally mineralize enough compounds, requiring a co-metabolism of compounds to degrade harmful wastes (Azubuike et al., 2016). Therefore, recent bioremediation studies have focused on microbial consortia rather than individual strains (Xu and Yu, 2021).

Some recent studies have demonstrated the possibility of using microbial consortia to reduce pesticide residues. A consortium formed by *Aspergillus versicolor* and bacteria isolated from sewage sludge (including *Pseudomonas, Klebsiella and Bacillus subtilis*) demonstrated greater efficiency degrading carbendazim and thiamethoxam compared with individual application (Rajpal et al., 2022). Additionally, a bacterial consortium comprised by *Pseudomonas plecoglossicida* and two isolates of *Pseudomonas aeruginosa* were shown to degrade organophosphate insecticides (Siripattanakul-Ratpukdi et al., 2014).

7.Microbial consortia in agriculture

In recent years, numerous studies have examined the use of microbial consortia in agriculture, encompassing fungi, bacteria, viruses, and even nematodes. Putative benefits of specific microbial selections for agriculture include plant disease control (Bo et al., 2022; Devi et al., 2022; De Vrieze et al., 2018; Fukui et al., 1999; Kim et al., 2008; Muthukumar et al., 2011; Slininger et al., 2007; Srinivasan and Mathivanan, 2009; Stummer et al., 2022), nematode control (Bo et al., 2022; Nafady et al., 2022; Siddiqui and Akhtar, 2009), arthropod control (Metwally et al., 2022; Monteiro et al., 2013; Soth et al., 2022) and/or plant growth promotion (Kaur et al., 2022; Kumawat et al., 2022; Muthuraja and Muthukumar, 2022).

In addition, microbial consortia can also be used to improve soils and mitigate abiotic stresses (Anshu et al., 2022; O'Callaghan et al., 2022) (Figure 2). Common genera in such consortia include *Trichoderma* (Stummer et al., 2022), *Bacillus* (Bouchard-Rochette et al., 2022), *Metarhizium* (Upamanya et al., 2020), *Beauveria* (Soth et al., 2022) and *Pseudomonas* (Kaur et al., 2022). Products comprising a variety of consortia are summarized in Table 1, 2 and 3 which show the synergistic or additive effects for various consortia.

7.1. Control of plant pathogens.

Many examples of microbial consortia developed in agriculture target plant diseases. Some studies suggest that microbial control of plant pathogens is improved with multiple strains of microorganisms containing different modes of action (Table 1).

Examples of synergism between different microbial taxa demonstrate multiple agricultural benefits. In tests with chickpea (*Cicer arietinum*), Siddiqui and Akhtar (2009) revealed that the combination of the filamentous fungus *Purpureocillium lilacinum* and soil nitrogen fixing bacteria *Rhizobium* sp. provides greater protection against root-knot nematode *M. javanica* and plant growth compared with strains applied separately. In this case, the authors proposed that while *P. lilacinum* parasitized female and eggs of *M. javanica*, antibiotics and phytoalexins produced by *Rhizobium* provided suppressive effects on the nematode. In tests with strawberry, Guetsky et al. (2007) observed that the combination of the yeast, *Pichia guilermondii*, and *Bacillus mycoides*, also exhibited a synergistic effect against the grey mold caused by *Botrytis cinerea*. In this case several mechanisms, including competition for nutrients and inhibition of germination and conidial damage, provided disease suppression. Through multiple regression analysis, the above-mentioned authors established that the mixture of *P. guilermondii* and *B. mycoides* provided additive activity, when contrasted with separate applications.

Some consortia targeting plant pathogens include botanical extracts. To control *M. incognita* on cucumber, Panpatte et al. (2021) investigated a consortium containing *Providencia vermicola* AAU PR1, and two *Pseudomonas* spp. *(P. putida* AAU PR2, and *P. fluorescens* AAU PR3), fortified with extracts of *Azadirachta indica, Ipomoea carnea* and *Brassica juncea*. In their study, highest suppression of *M. incognita* eggs was observed with the fortified consortium, which the authors linked to the synergistic effect of the botanical extracts with bacterial strains. In tests with cultivated rice, Kaewsalong et al. (2019) verified that the combination of strains of *T. harzianum* and *T. asperellum* plus *Coscinium fenestratum* extract

was effective in controlling rice diseases induced by *Alternaria padwickii, Curvularia lunata, Fusarium moniliforme* and *Bipolaris oryzae*. Other examples to control of plant diseases with consortia are show in Table 1.

7.2. Use of Trichoderma in consortia.

The ubiquitous soil fungi *Trichoderma* spp. have become widely produced and developed for commercial use in agriculture to control of plant diseases (Lorito and Woo, 2015; Monte and Hermosa, 2021; Woo et al., 2014). *Trichoderma* controls plant pathogens directly via parasitism, competition, antibiosis, and the production of enzymes, volatile organic compounds, and secondary metabolites and indirectly through the induction of resistance (Lorito and Woo, 2015; Monte and Hermosa, 2021; Woo et al., 2014). Although primarily used to suppress root diseases, *Trichoderma* can provide additional functions, including increasing nutrient availability through mineralization and solubilization, and improving soil structure and plant growth (Bononi et al., 2020; Lorito and Woo, 2015; Monte and Hermosa, 2021; Woo et al., 2014).

Globally, many products are formulated with *Trichoderma* spp. Bettiol et al. (2019) reported 246 registered products containing *Trichoderma*. Among these, 28% were formulated with mixed *Trichoderma* species strains or with other microorganisms, including *Arthrobacter*, *Azotobacter*, *Bacillus*, *Pseudomonas*, *Azospirillum*, *Rhizobium*, *Beauveria*, *Metarhizium*, *Saccharomyces*, *Purpureocillium*, *Streptomyces*, *Agrobacterium radiobacter*, and *Pochonia chlamydosporia* (Table 4).

7.3. Control of arthropod pests.

The use of microbial pathogens against insects was first reported at the end of the last century, when the potential of *M. anisopliae* s.l. to control Japanese beetles (*Popilia japonica*) was demonstrated (Faria, 2001). In recent years entomopathogenic microbes applied in isolation have been used to control a suite of arthropod pests (Aynalem et al., 2022; Biryol et al., 2022; Oliveira et al., 2022; Stuart et al., 2020). However, several studies have shown that different microbes mixed together can provide enhanced control, through increase in virulence-associated metabolites, and other molecules with antioxidant and anti-inflammatory properties. Such components can supress the insect immune system and facilitate the infection by

entomopathogenic microbes (Stuart et al., 2020/2022; Metwally et al., 2022; Nawaz et al., 2020; Soth et al., 2022; Wu and Duncan, 2022).

There are several examples of microbes applied together improving control of insect pests compare with single strain applications (Table 2). When applied individually, four species of *Beauveria* exhibited relatively low virulence. However, when combined as a consortium, these species induced 100% mortality in *Plutella xylostella*, indicating a synergistic effect (Soth et al., 2022). Stuart et al. (2022) demonstrated that a consortium of two strains of *B. bassiana* (Bov 2 and Bov 3) exhibited increased virulence against *Duponchelia fovealis* larvae, compared with either strain in isolation. In this case, increased chitinase and lipase activities of the mixture, identified through a metabolomic approach, were hypothesized to suppress host immunity, increasing its susceptibility to fungal infection.

In the field, entomopathogens applied for pest control may compete with native or resident strains of other microbes. Using microsatellite molecular markers, Iwanicki et al. (2019) tracked the fate of the ESALQ1604 strain of *M. anisopliae* applied against spittlebug *Mahanarva* spp. in sugarcane. Results showed that native strains of *M. anisopliae* accounted up to 50% of all infected insects recovered with this fungus, which contributed significantly to the overall biocontrol of this pest. The use of molecular markers to track the fate and persistence of entomopathogens applied in the field is a useful tool to understand their interactions with native microbes, since it remains unclear how such interactions influence biological control.

There are relatively few reports of microbial consortia containing more than two phyla. Spescha et al. (2023) evaluated a soil bacterium (Pseudomonas chlororaphis) nematode (Steinernema feltiae) and fungus (Metarhizium brunneum) against the soil dwelling pest, the cabbage maggot *Delia radicum. Based on laboratory, greenhouse and field studies, the authors observed that all* three species were compatible and did not inhibit each other's infectivity nor survival in the soil or roots. Moreover, they concluded that synergistic effects of bacteria-nematode and bacteria-fungus combinations were possible.

7.4. Improving plant growth and mitigation of abiotic stress.

Plant biostimulants, also known as biofertilizers or plant enhancers, include a range of compounds or products claimed to promote plant growth, independently of their nutritional content (Tarigan et al., 2022). Many biostimulants contain microorganisms, which may also provide some plant protection properties. Studies have demonstrated the potential of microbial consortia to increase plant growth or yield by combining microorganisms known to solubilize

nutrients, fix nitrogen, and produce phytohormones and siderophores (Behera et al., 2021; Chen et al., 2010; Comite et al., 2021; Kaur et al., 2022; Lanzuise et al., 2022; Yadav et al., 2022) (Table 3).

Recent studies suggest that using microbial consortia provides significant plant growth benefits, when compared with single species inoculations. Kumawat et al. (2022) evaluated a soil bacterium *Bradyrhizobium* sp. LSBR-3 with phosphate-solubilizing *Pseudomonas oryzihabitans* LSE-3 in soybean. It was found that plants treated with the duel inoculants showed improvements in several beneficial traits (including root growth and nutrient accumulation) compared with single inoculation, and provided an 11% yield increase compared with control plots. Furthermore, a consortium of four mycorrhizal fungi (*Funneliformis constrictum, F. mosseae, Gigaspora margarita* and *Rhizophagus irregularis*) and *B. bassiana* promoted cotton growth, increased protein and carbohydrates contents and decreased *Spodoptera littoralis* growth parameters (Metwally et al., 2022).

Additional studies demonstrate the potential of microbial consortia to mitigate plant abiotic stress (Basu et al., 2022). Examples include enhanced tolerance to salt (Anshu et al., 2022; Dixit et al., 2023; Wilmowicz et al., 2022; Xu et al., 2022), drought (Ouhaddou et al., 2023) and heavy metal stresses (Shilev et al., 2020).

7.5.Soil health.

Microbial activity is essential for soil health (Fierer et al., 2021). For example, soils with high microbial biomass have a great potential to accumulate and cycle organic and inorganic nutrients (Gregorich et al., 2011). Microorganisms also play an important role in soil biogeochemical processes, including lignin and cellulose degradation and soil carbon storage (Lacerda-Júnior et al., 2017/2019; Lei et al., 2018; Sun et al., 2017).

The application of microbial consortia to promote soil health is still relatively new and understudied. However, early research suggests that this approach may provide multiple benefits, including improving soil fertility, nutrient uptake, and crop yields (Anshu et al., 2022; Lei et al., 2018; Shukla et al., 2020; Sun et al., 2017; Vishwakarma et al., 2020). For example, applications of the fungi *Trichoderma harzianum*, *Gluconcetobacter diazotrophicus*, and *Pseudomonas fluorescens* ($10^{9–10}$ CFU/mL liquid culture) in sugarcane resulted in a 50% reduction in NPK requirements, due to greater cation exchange capacity between soil and roots (Shukla et al., 2020). Bacterial consortia can also be used to accelerate composting of green wastes by stimulating lignocellulose degradation (Oviedo-Ocaña et al., 2022).

8. Multifunctionality.

Some microbial consortia provide multiple benefits. Senthilraja et al. (2010) reported the efficacy of a consortium containing *B. bassiana* B2 and *P. fluorescens* (TDK1 and Pf1) in controlling both groundnut leaf miner (*Aproaerema modicella*) and southern blight [*Athelia* (=*Sclerotium rolfsii*) while promoting the growth of peanut plants. Similarly, Silva et al. (2022) reported the multifunctional potential of two *Trichoderma* strains (*T. asperelloides* CMAA 1584 and *T. lentiforme* CMAA 1585) to control *S. sclerotiorum* and to increased cotton plant growth. While the CMAA 1584 strain demonstrated efficiency in controlling *S. sclerotiorum*, the CMAA 1585 strain promoted cotton plant growth and helped solubilize inorganic phosphates. Favaro et al. (2022) described a granular product containing a mixture of *T. asperelloides* (biocontrol agent) and *Aspergillus niger* (phosphorus solubilizer). The granules were shown to solubilize inorganic phosphorus and to inhibit the growth of *Fusarium oxysporum*.

9.Adverse cases

While artificial combinations of microorganisms can provide synergistic or otherwise beneficial effects compared with single strains, this is not always the case. For example, applications of *B. velezensis* BMH + INV strains provided greater suppression of *M. incognita* galls and eggs in tomato roots when applied separately, compared with their combination (Cruz-Magalhães et al., 2022). The authors observed the BMH strain produced fewer volatile compounds when mixed with the INV strain. Similar results were reported by Stockwell et al. (2010), when a bacterial consortium of *Pantoea fluorescens* A506, *P. vagus* C9–1 and *P. agglomerans* Eh252 generally provided lower control of *Erwinia amylovora* in pear compared with bacteria applied individually. Felici et al. (2008) also found that co-inoculation of *B. subtilis* 101 and plant growth promoting rhizobacterium *Azospirillum brasilense* Sp245 failed to increase plant biomass, whereas individual applications of these microorganisms did. Taken together, these examples highlight complexities of potential interactions between microorganisms applied in consortia.

10.Commercial microorganisms formulated in consortia: Brazilian case

Brazil is the largest global producer and user of biocontrol agents with > 70 million hectares treated in 2022 (Medeiros and Bettio, 2023). The larger area under biological control reflects long term cultivation, its climatic conditions and the large number of registered biopesticides (more than 480 in 2022). Brazil's agricultural production plays a pivotal role in global food security with almost 42 million hectares of soybean, 22 million hectares of corn, 9 million hectares of sugarcane, and 2 million hectares of coffee among other crops (Medeiros and Bettiol, 2023).

Many biological products registered in Brazil contain a mixture/consortium of microorganisms or strain combinations. Among the 480 biopesticides products to control pests and plant diseases registered in Brazil, 83 products were formulated with combinations of microorganisms, mainly registered in the last three years. The main species used in mixtures to control plant diseases include *B. amyloliquefaciens* (22.9%), *B. subtilis* (12.1%), *B. licheniformis* (7.2%), *B. velezensis* (4.9%), *T. harzianum* (12.1%), *B. bassiana* (8.5%), *M. anisopliae* (8.1%), *T. viride* (6.3%), *Paecilomyces lilacinus* (5.4%), and 17.5% with ten other species (AGROFIT - Sistema de Agrotóxicos Fitossanitário, 2023) (Table 5). Bionematicides comprise 19 products, comprising seven strains, with 75% of the products formulated with *B. subtilis* (32.8%), *B. licheniformis* (26.2%), *Purpureocillium lilacinum* (19.7%), *B. amyloliquefaciens* (8.2%), *Bacillus paralicheniformis* (6.5%), *B. thuringiensis* (4.9%), and *B. velezensis* (1,6%) (Figure 3). These products included bioinsecticides (32%), biofungicides (41%), bionematicides (22%), and biofungicides/bionematicides (5%) (Figure 3).

Actually, the combination of strains may be designed based on many of the strategies proposed by academia and mentioned in the topics above, i.e. niche complementarity, additive metabolic profile, different optimum temperature, different benefits to the plants exerted exclusively by individual strains and combined in one single product. For instance, combining a microbial strain with antimicrobial activity with a soilborne biocontrol agent or a combination of more than one of such strategies. There is a product on the Brazilian marked that encompasses three strains: *Bacillus subtilis* CCTB04, *Bacillus velezensis* CCTB09 and *Bacillus pumilus* CCTB05. Although the rationale of combining those specific strains is not always mentioned by the companies as a commercial secrete not to be copied, other display such benefits but they are not endorsed by peer reviewed publication. A third group encompass strains selected by the academia, for which their reputation had previously been reported. In the example above, the potential of the individual *Bacillus* strains exerts benefits related to disease control (Ferreira,

2021) and growth promotion (Venancio et al., 2019) is reported and it is important to mention that we need to encompass previous taxonomic denominations of the same bacterial strain to retrieve all reported benefits it exerts.

However, such product is traded as bioprotectant only, since there is no possibility of registering a product that exert growth promotion and bioprotection. Actually, there are different regulations to register biologicals in the world and to the best of our knowledge none of them consider both benefits in one single product. In Brazil for instance, they can be registered as biofertilizer or bioprotectant. The industry has to decide on registering a product following the biofertilizer regulatory framework if the microorganism exert a growth promotion, nutrient fixing or solubilizing benefit to the plant. On the other hand, they choose the biopesticide route if the microorganisms exert any action direct disease or pest control. Here comes what we call the regulatory trap. A single microorganism can exert both a growth promotion and a disease/pest control benefit but there is no possibility of registering a product that has both benefits. This is even worse when one strain exerts a biofertilizer-type benefit and another one a biocontrol potential. Therefore, most companies decide on the reductionist microbial registration to one benefit only and adopt a technical recommendation that the products should be combined in one single application as tank mix or seed treatment slurry, i.e. a biofertilizer-type product (e.g. nitrogen-fixing bacteria) combined with a biocontrol-type one (e.g. soilborne disease control).

11.Conclusions, challenges and perspectives

The use of ‘microbial consortia’ in agriculture is a reality, as evidenced by the growth in the global supply of biological products containing multiple species/strains. It is clear that potential benefits can result from careful selection of microbial mixtures, allowing potential improvements in the efficiency, reliability and consistency of applications. Microbes may also help reduce the use nitrogen fertilizers based on fossil fuels, and contribute to carbon sequestration through mutualistic associations with plants. It is noteworthy that some microbial consortia may alleviate drought stresses, already considered a limiting factor in crop production in many regions. We anticipate an increased contribution of microbial consortia in sustainable agricultural production systems. Importantly, with the anticipated growth in biopesticides and biostimulants, several companies are likely to invest more resources in this technology. As a

result, we envision that microbial consortia will continue to grow, bringing environmentally friendly solutions to cope with current hurdles faced in contemporary agricultural systems.

Although an increasing number of research publications address this topic, detailed field studies are still limited. This review discusses the recent applications and future perspectives of microbial consortia in sustainable agriculture, with a special focus on phytosanitary management. Additionally, it highlights the need for research to hain deeper understanding of the intricate interactions between different players and native microbial communities in a broader environment. Future studies using qPCR and molecular probes, including metagenomic and amplicon sequencing, may be helpful tools to track the establishment and/or persistence of specific microbes in the field and decipher the mechanisms of plant-microbe interactions. Through such approaches, a more general model of microbial consortia for specific crops/applications may emerge.

The future of synthetic microbial consortia in plant care and protection holds considerable promise, but it also faces several challenges. Here's an overview of both aspects:

12.Future Prospects:

1-Precision Agriculture: Synthetic microbial consortia can be customized for specific crops and environmental conditions, enabling precision agriculture. Tailoring microbial communities to meet the unique needs of different plants may enhance overall agricultural productivity. **2-Holistic Plant Health Management:** By incorporating diverse microorganisms with complementary functions, synthetic consortia can provide holistic solutions for plant health management. This includes biocontrol of pathogens, stimulation of growth, and adaptation to environmental stress. **3-Resilience to Climate Change:** Engineered microbial consortia may help plants adapt to changing climate conditions, offering resilience against temperature fluctuations, water scarcity, and emerging diseases. **5-Reduced Environmental Impact:** The use of synthetic microbial consortia aligns with sustainable agricultural practices by reducing the reliance on chemical inputs, minimizing environmental impact, and promoting regenerative agriculture. **6-Enhanced Nutrient Cycling:** Microbial consortia can contribute to nutrient cycling and soil health, promoting sustainable agriculture by reducing the need for chemical fertilizers.

Challenges:

1-Complexity of Microbial Interactions: Understanding and engineering interactions within synthetic microbial consortia is a complex task. Predicting how different species will behave in a community and ensuring stability over time pose significant challenges. Understanding the

compatibility between species of microorganisms, including potentially synergistic and antagonistic/competitive interactions under operational conditions will essential. **2-Regulatory Approval:** The regulatory landscape for synthetic microbial products in agriculture is still evolving. Obtaining approvals for the commercial use of synthetic consortia may be hindered by regulatory uncertainties and safety concerns. **3-Scalability and Production Challenges:** Scaling up the production of synthetic microbial consortia for large-scale agriculture presents logistical and economic challenges, improving the global supply chain and technology transfer to farmers. Maintaining consistency in microbial formulations and ensuring cost-effective production are crucial factors. **4-Long-Term Stability:** Ensuring the long-term stability and performance of synthetic consortia in diverse field conditions is essential. Environmental factors, competition with native microbes, and other unpredictable variables could impact their efficacy over time. **5-Ethical and Societal Concerns:** The release of synthetic organisms into the environment raises ethical and societal concerns. Balancing the potential benefits with potential unintended consequences and addressing public perception is critical. It will be necessary quantify the effects on plant health, including indirect protection against pests and diseases compared with current agrochemical interventions. **6-Interdisciplinary Collaboration:** Successful implementation of synthetic microbial consortia requires collaboration between microbiologists, geneticists, agronomists, and regulatory bodies. Bridging gaps between these disciplines is necessary for effective development and application.

In summary, the future of synthetic microbial consortia in plant care and protection is promising, offering innovative solutions for sustainable agriculture. However, addressing the associated challenges will be crucial to realizing their full potential and ensuring responsible deployment in the agricultural landscape.

Acknowledgements

PSON acknowledges Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the scholarship. WB and FHVM are supported by a productivity fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 307855/2019-8 and CNPq 317266/2021-7, respectively).

Author contributions

All authors contributed similarly. All authors read and approved the final manuscript.

Conflict of interest statement

All authors declare that there is no conflict of interest in this review.

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Fig. 1. Incompatibility between microorganisms by the paired culture method. A: *Cordyceps javanica* (CMAA1228) and *Bacillus velezensis* (AP03); B: *Beauveria bassiana* (CMAA 1807) and *B. velezensis* (AP03); C: Beauveria caledonica (CMAA 1810) and *B. velezensis* (AP03); D: *Trichoderma asperelloides* (CMAA 1584) and *B. velezensis* (AP03); E: *C. javanica* (CMAA1228) and *B. bassiana* (CMAA 1807); F: *T. asperelloides* (CMAA 1584) and *B. bassiana (*CMAA 1807). From A to D distance inhibition; E and F invasion or replacement. Interactions evaluated according to Mohammad et al. (2011).

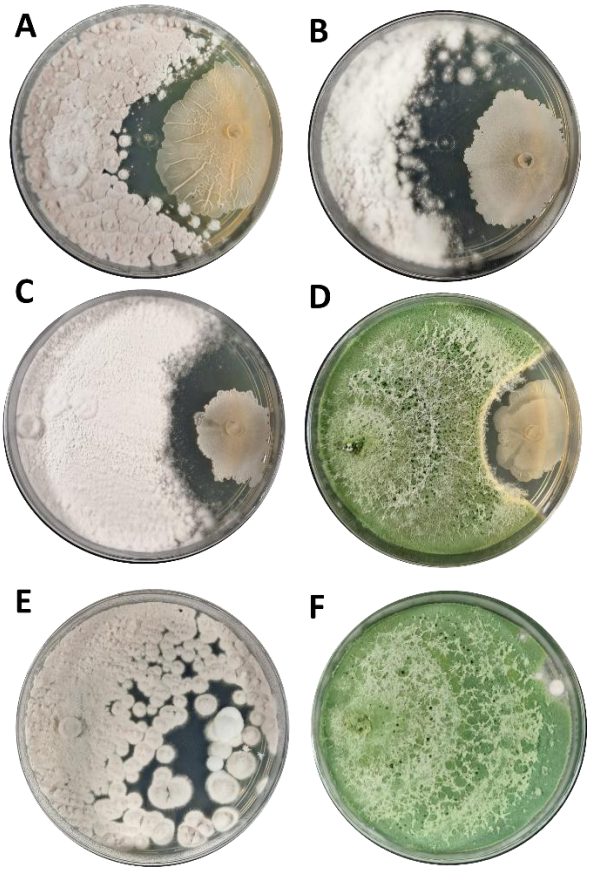

Fig. 2. Effects of the application of consortia of microorganisms.

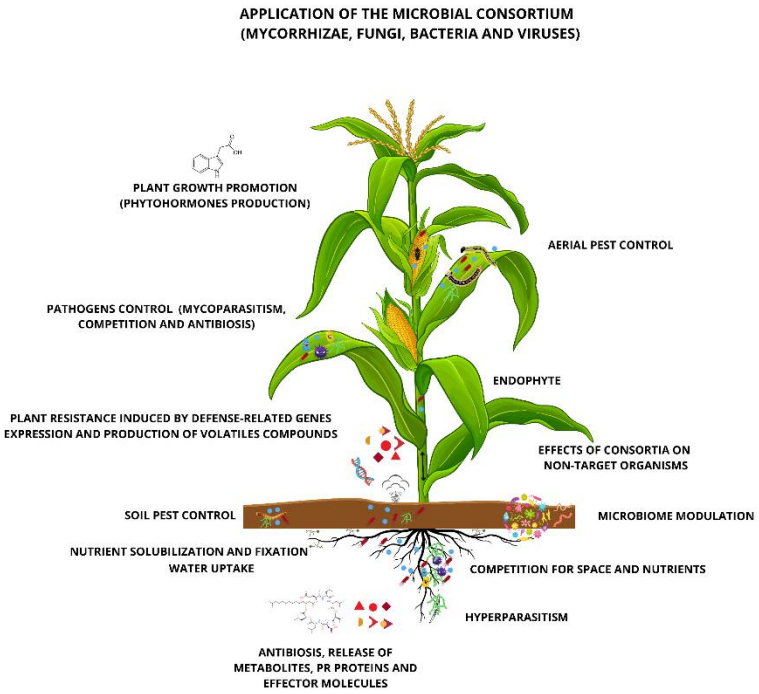

Fig. 3. Main species and strains used in biological products, registered in Brazil, containing microorganism consortia. Data obtained of official site of Agriculture Minister (https://agrofit.agricultura.gov.br/) accessed in 06/02/2023.

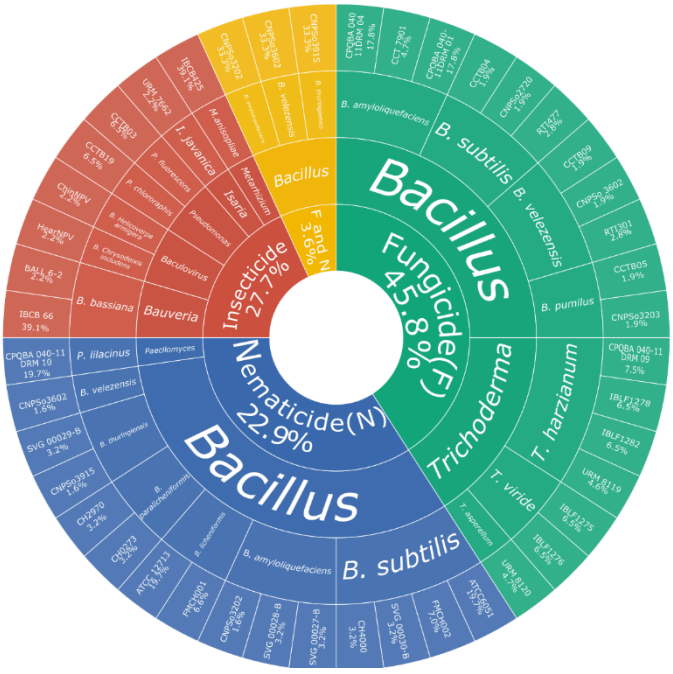

Table 1. Microbial consortium to control of plant diseases more effectively than antagonists individually.

Microbial consortium	**Pathogen**	**Mode of action**	**Effect**	**Culture**	**Reference**
Sphingomonas paucimobilis, Microbacteria sp., *Brevundimonas vesicularis, Pseudomonas rubrisubalbicans* and *Alcaligenes eutrophus*	*Xanthomonas campestris* pv. *dieffenbachiae*	Competition for nutrients	Synergism	Anthurium	Fukui et al. (1999)
Pseudomonas fluorescens (strains S22:T:04, P22:Y:05, S11:P:12) and *Enterobacter cloacae* (S11:T:07)	*Phytophthora infestans*	Uninformed	Synergism	Potato	Slininger et al. (2007)
Serratia plymuthica (C-1), *Chromobacterium* sp. (C-61) and *Lysobacter enzymogenes* (C-3)	*Phytophthora capsici*	Enzyme production and antibiosis.	Synergism	Pepper	Kim et al. (2008)
*Bacillus licheniformis (*MML2501*)*, *Bacillus* sp. (MML2551), *Pseudomonas aeruginosa (*MML2212*)* and *Streptomyces fradiae (*MML1042)	Sunflower necrosis virus disease (SNVD)	Resistance induction and growth promotion	Synergism	Sunflower	Srinivasan and Mathivanan (2009)

Pseudomonas fluorescens (Pf1) and *Beauveria bassiana* (AH1)	*Rhizoctonia solani*	Resistance induction	Synergism	Rice	Karthiba et al. (2010)
Trichoderma viride (TVA) and *P. fluorescens* (EBL 20-PF)	*Pythium aphanidermatum*	Activation of the defense mechanism	Synergism	Pepper	Muthukumar et al. (2011)
Trichoderma sp., *Bacillus* sp. and *P. fluorescens*	*Sclerotinia sclerotiorum*	Resistance induction	Synergism	Pea	Jain et al. (2012)
Pseudomonas spp. (R32, R47, R76, R84, S34, S49, S35, S04 e S19).	*P. infestans*	Inhibition of zoospore formation	Additive	Potato	De Vrieze et al. (2018)
B. subtilis (znjdf1) and *T. harzianum* (znlkhc1)	*Streptomyces* spp.	Promotes the recruitment of beneficial bacteria in the potato rhizosphere.	Synergism	Potato	Wang et al. (2019)
T. harzianum, *B. bassiana* and *Metarhizium anisopliae*	*Phomopsis*, *Alternaria* and *Fusarium*.	Growth promotion, suppressiveness increase and resistance induction.	Synergism	Eggplant	Upamanya et al. (2020)
Trichoderma gamsii (A5MH) and *T. harzianum* (Tr906)	*Fusarium pseudograminearum*	Growth promotion, antibiotic production, and niche competition.	Additive	Wheat	Stummer et al. (2022)

Funneliformis mosseae, *Glomus fasciculatum* and *Bacillus* sp.	*Fusarium oxysporum* f. sp. *lycopersici*	Production of lytic enzymes, and siderophore, antibiosis, and phosphate solubilization.	Synergism/ Additive	Tomato	Devi et al. (2022)
Bacillus pumilus (PTB180) and *B. subtilis* (PTB185)	*Botrytis cinerea*	Production of surfactin, iturine and fengycin.	Additive	Tomato	(Bouchard-Rochette et al. (2022)
Bacillus nematocida (B16) and *Pochonia chlamydosporia* (ZK7)	*Meloidogyne incognita*	Production of volatile substances, proteases and chitinases, efficient root colonization.	Synergism	Tomato	Bo et al. (2022)
Trichoderma harzianum (MZ025966) and arbuscular mycorrhizal fungi	*Meloidogyne javanica*	Induction of systemic resistance, growth promotion and increased enzymatic activity	Synergism	Tomato	Nafady et al. (2022)
*Metarhizium brunneum (*KVL 16–36), *M. robertsii (*KVL 12–35), *B. bassiana (*KVL 13–39) and *Funneliformis mosseae* (BEG12).	*Botrytis cinerea*	Growth promotion and systemic changes in plant defense.	Synergism	Tomato	Zitlalpopoca-Hernandez et al. (2022)

Table 2. Microbial consortium to control of insect's pest more effectively than bioagents individually.

Microbial consortium	**Insect pest**	**Mode of action**	**Effect**	Reference
Beauveria bassiana and *Metarhizium flavoviride*	*Melanoplus sanguinipes*	Overcoming temperature constraints in Hyphomycetes	Synergism	Inglis et al. (1997)
B. bassiana (Bb 9001, Bb 9005, Bb 9010, Bb 9011, Bb 9119, Bb 9205, Bb 9016, Bb 9020, Bb 9023, Bb 9024)	*Hypothenemus hampei*	Uninformed	Synergism/Additive	Cruz et al. (2006)
Pseudomonas fluorescens (Pf1) and *B. bassiana* (AH1)	*Cnaphalocrocis medinalis* and *Rhizoctonia solani*).	Enzyme accumulation, lipoxygenase and chitinase activity	Synergism	Karthiba et al. (2010)
P. fluorescens (Pf1 e TDK1) and *B. bassiana* (B2)	*Aproaerema modicella* and *Sclerotium rolfsii*	Increase enzymatic activity and phenolic compounds	Synergism	Senthilraja et al. (2013)
Heterorhabditis bacteriophora (HP88), *Heterorhabditis indica* (LPP1), *Metarhizium anisopliae* (IBCB116) and *B. bassiana* (986)	*Rhipicephalus microplus*	Mechanisms are not understood	Synergism	Monteiro et al. (2013)

B. bassiana (B2) and *Bacillus subtilis* (EPC8)	*Helicoverpa armigera* and *Fusarium oxysporum* f. sp. *lycopersici*	Accumulation of defense enzymes such as lipoxygenase, peroxidase and polyphenol oxidase	Synergism	Prabhukarthikeyan et al. (2014)
*Metarhizium brunneum (*KVL 04–57), *M. flavoviride (*KVL 14–112), *M. robertsii (*KVL 12–35) and *Clonostachys rosea (*IK726)	*Tenebrio molitor* and *Fusarium culmorum*	Mechanisms are not understood	Synergism	Keyser et al. (2016)
Steinernema and *Heterorhabditis*	*Galleria mellonella*	Predation	Synergism/Additive	Wu and Duncan (2022)
Trichoderma sp. and *Bacillus thuringiensis*	*Aphis gossypii* and *Amrasca bigutulla bigutulla*	Predation	Synergism/Additive	Nawaz et al. (2020)
*B. bassiana (*Bea 111, Bov 2 e Bov 3), *Isaria javanica* (Isa 340) and *Purpureocillium lilacinum* (Pae 69)	*Duponchelia fovealis*	Production of lytic enzymes.	Synergism	Stuart et al. (2020)
B. bassiana (Bea 111, Bov 2 e Bov 3), *I. javanica* (Isa 340) and *P. lilacinum* (Pae 69)	*Duponchelia fovealis*	Production of molecules with antioxidant and anti-inflammatory, toxin, and growth promotion.	Synergism	Stuart et al. (2022).

B. bassiana, B. caledonica, B. malawiensis and *B. pseudobassiana*	*Plutella xylostella*	Uninformed	Synergism/Additive	Soth et al. (2022)
B. bassiana e *Funneliformis mosseae, Funneliformis constrictum, Gigaspora margarita* and *Rhizophagus irregularis*)	*Spodoptera littoralis*	Production of nematicidal molecules; growth promotion and detoxification plants.	Synergism	Metwally et al. (2022)
Pseudomonas chlororaphis (PCLRT03, PCLRT03-gfp and PCLRT03-mturq), *Steinernema feltiae* (RS5), *Xenorhabdus bovienii* (SM5) and *M. brunneum* (Bip5 and Bip5-gfp).	*Pieris brassicae* and *Diabrotica balteata*	Production of insecticidal and antimicrobial toxins and compounds that modulate and suppress the insect immune response.	Synergism	Spescha et al. (2023)

Table 3. Microbial consortium to promote growth and reduce plant stress more effectively than bioagents individually.

Microbial consortium	**Mode of action**	**Effect**	**Culture**	**Reference**
Trichoderma asperellum (GDFS1009) and *Bacillus amyloliquefaciens* (1841)	Increase seedling emergence. Expression of 1-aminocyclopropane-1-carboxylate (ACC) deaminase.	Synergism	Wheat	Karuppiah et al. (2019)
Pseudomonas putida, *Pseudomonas fluorescens, Bacillus thuringiensis* and *Pseudomonas synxantha*	Decreased Cd, Pb and Zn accumulation throughout the plant and increase plant biomass.	Synergism	Spinach	Shilev et al. (2020)
Brevibacterium halotolerans (Sd-6), *Bacillus subtilis* (Ldr-2), *Achromobacter xylosoxidans* (Fd-2), *Burkholderia cepacian* (Art- 7) and *Trichoderma harzianum* (Th)	Increase accumulation of proline, starch, total phenolics, and reduced the accumulation of 1-aminocyclopropane-1-carboxylic acid. Cold stress alleviation.	Synergism/Additive	Basil	Singh et al. (2020)

T. harzianum, Beauveria bassiana and *Metarhizium anisopliae*	Production of growth hormones secreted by organisms, increasing nutrient and moisture availability	Synergism	Eggplant	Upamanya et al. (2020)
T. harzianum (OMG16) and *Bacillus* spp.	Mitigation of cold stress and reduction of stress priming effects.	Synergism	Corn	Moradtalab et al. (2020)
Trichoderma atroviride (LX-7), *Trichoderma citrinoviride* (HT-1)	Indoleacetic acid (IAA)and siderophores production	Synergism	Pak - Choi	Chen et al. (2021)
Bacillus sp. (CP4 e AHP3) and mycorrhizal fungi	Higher concentrations of proline.	Synergism	Wheat	Yadav et al. (2022)
Piriformospora indica (DSM 11827 and *Azorhizobium caulinodans* (B81176)	Promoting growth and mitigating saline stress. Increase superoxide dismutase, catalase, peroxidase activities.	Synergism	Tomato	Xu et al. (2022)
Bacillus licheniformis (MN718157) and *Aspergillus violaceofuscus* (MH220545)	Improve catalase activity and proline content.	Synergism	Tomato	Muthuraja and Muthukumar (2022)

Erwinia sp. (EU-B2SNL1), *Chryseobacterium arthrosphaerae* (EU-LWNA-37) and *Pseudomonas gessardii* (EU-MRK-19)	N fixation and mineral solubilization	Synergism	Barley	Kaur et al. (2022)
Trichoderma koningiopsis NBRI-PR5 (MTCC 25372) and *T. asperellum* NBRI-K14 (MTCC 25373)	Growth promotion under saline stress conditions.	Synergism	Rice	Anshu et al. (2022)
Glomus spp. and *Bacillus* sp.	Reduction of water stress.	Synergism	Corn	Wilmowicz et al. (2022)
Beauveria bassiana, Funneliformis mosseae, Funneliformis constrictum, Gigaspora margarita and *Rhizophagus irregularis*	Detoxification mechanism in plants under stress. Increase enzymes activities and in proline.	Synergism	Cotton	Metwally et al. (2022)
Glomus versiforme and *Bacillus methylotrophicus*	Increased chlorophyll, carotenoids, photosynthesis. Drought tolerance.	Synergism	Tabacco	Begum et al. (2022)
Macrophomina pseudophaseolina, Paraphoma radicina, Trichoderma	Drought tolerance. Increase in soil enzymes to promote N and P uptake.	Synergism	*Astragalus mongholicus*	Li et al. (2022)

afroharzianum, Trichoderma longibrachiatum				
Bacillus safensis NBRI 12M, *B. subtilis* NBRI 28B, NBRI 33N, NBRI 53L, *Jeotgalicoccus huakuii* NBRI 13E, *B. stratosphericus* NBRI 7[a]	Protect from salinity stress.	Synergism/Additive	Arabidopsis	Dixit et al. (2023)

Table 4. Products formulated with a mixture of *Trichoderma* and other microorganisms of active ingredients registered and marketed worldwide.

Product	Active Ingredient	Biological target	Commercialization/Company
Biotam WP	*Trichoderma asperellum* ICC 012 and *Trichoderma gamsii* ICC 080	*Armillaria*, *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Sclerotinia*, *Sclerotium*, *Thielaviopsis*	USA - Isagro
Bioten WP	*T. asperellum* ICC 012 and *T. gamsii* ICC 080	*Rhizoctonia solani*, *Sclerotinia*, *Verticillium dahliae*, *Thielaviopsis basicola*; *Phytophthora capsici*	EU and USA - Bayer CropScience
Remedier and Tenet	*T. asperellum* ICC 012 and *T. gamsii* ICC 080	*Pythium*, *Phytophthora*, *R. solani*, *Sclerotinia*, *S. rolfsii*, *T. basicola*, *V. dahliae*; *Armillaria mellea*	EU and USA - Isagro
Kiwivax	*Trichoderma atroviride* LU668 and LU297, *T. virens* LU753	*Pseudomonas syringae* pv. *actinidiae*	New Zeeland - Agrimm

Promot	*T. koningii* and *T. harzianum*	*Phythophtora, Sclerotinia, Botrytis, Rhizoctonia, Pythium, Fusarium*; *Phomopsis*	Germany - Biofa AG
Trichogel	*T. harzianum* and *T. koningii*	*R. solani*	Colombia - Soluciones Microbianas
Tricox	*T. koningii* and *T. harzianum*	*Meloidogyne incognita* and *Phythophtora capsici*	Peru - JH Biotech
Tricotop	*T. harzianum* and *T. koningiopsis*	*Rhizoctonia, Fusarium, Pythium*	Bolivia - BioTop
Binab T	*T. polysporum* IMI 206039 and *T. harzianum* IMI 206040	*Chondrostereum, Verticillium, Rhizoctonia, Fusarium, Phomopsis, Sclerotium, Sclerotinia*; *Pythium, Botrytis, Heterobasidium, A. mellea*	UE and USA - Binab Bio-Innovation
RootshielD	*T. harzianum* T-22 and *T. virens* G-41	*Pythium, Rhizoctonia, Fusarium, Thielaviopsis, Phytophthora*, and *Cylindrocladium*	EU, USA and Canada -BioWorks

Fertimax	*T. viride* and *T. harzianum*	*Pythium, R. solani, Fusarium, B. cinerea, S. rolfsii, Sclerotinia homoeocarpa*; *Ustilago tritici*	India - Skymax Crop Science
Poabs Green	*T. viride* and *T. harzianum*	*Phytophthora capsici*, *Pythium, F. oxysporum, R. solani, Rosellinia arcuata*; nematodes	India - Poabs Biotech
Radix WP	*T. harzianum* ICC 012 and *T. viride* ICC 080	*R. solani, S. sclerotiorum, V. dahliae, T. basicola*; *P.capsici*	Italy - Certis
Trich-A-Soil	*T. harzianum* and *T. viride*	*Fusarium*; *Pythium spinosum.*	Australia - Organic Crop Protectants
Valery Plus	*T. harzianum* and *T. viride*	*B. cinerea*.	Peru - Agrobioticos
Nutri-Life Tricho-Shield	*T. harzianum, Trichoderma lignorum* and *Trichoderma koningii*	Plant growth promoter	Australia - Biological Solutions
Trichonativa	*T. harzianum, T. virens* and *Trichoderma parceramosum*	*Borytis, Alternaria, Venturia, Phytophthora, Fusicoccum, Verticillium, Sclerotinia*; *C. purpureum*	Chile - BioInsumos Nativa

3 TAC	*T. viride, T. harzianum* and *Trichoderma longibrachiatum*	*Fusarium, Pythium, Alternaria, Botrytis, Erysiphe, Sclerotinia, Bremis, Phytophthora, Septoria, Cercospora, Leiveillula taurica, P. syringae*	Chile and Peru - Pinturas Renner, Avance Biotechnologies
Trichonativa	*T. viride*, *T. harzianum* and *T. longibrachiatum*	*P. capsici, Pythium, R. solani, Sclerotinia, B. cinérea*.	Chile - BioInsumos Nativa
Fitotripen	*T. harzianum, T. koningii* and *T. viride*	*R. solani*, *Phytophthora*; *Fusarium*	Colombia - Natural Control
Tribiol	*T. harzianum, T. koningii* and *T. viride*	*R. solani*.	Colombia - Bioprotección
Custom	*T. harzianum, T. viride, T. koningii, T. polysporum.*	Soil probiotic	USA - Custom Biologicals
Safersoil	*T. asperellum, T. atroviride, T. harzianum*; *P. lilacinus*	*R. solan*i; *Meloidogyne*	Colombia - Safer Agrobiológicos
Shakti	*T. harzianum, T. viride* and *P. lilacinus*	Wilt, damping off, *Ganoderma*; Nematodes	India - Nivshakti

Trombo	*T. harzianum* and *Saccharomyces cereviseae*	Composting	Colombia - Bio-Crop
Tropimezcla	*Trichoderma, B. bassiana, M. anisopliae, P. lilacinus, S. cereviseae*	*Alternaria*	Colombia - Soluciones Microbianas
Annapurn	*Trichoderma*, *Azotobacter*, *Azospirillum*, *Rhizobium*; *Pseudomonas*	Soilborne pathogens	India - Multiplex Group
Anoka	*T. viride* and *P. fluorescens*	*Pythium, Phytophthora, Rhizoctonia, Fusarium*	India - KN Bio Sciences
Biotamax, Biotragreen	*T. harzianum, T. viride, T. koningii, T. polysporum, Bacillus laterosporus, B. licheniformis, B. megaterium, B. pumilus, B. subtilis* and *Paenibacillus polymyxa*	Inoculant and biofertilizer.	USA - Custom Biologicals
Bio Effekt	*T. viride, T. lignorum* and *B. subtilis*	*Fusarium, Helminthosporium, Rhizoctonia, Pythium,*	Russia - Biona (Биона)

		Sclerotium, Alternaria, Verticillium; *Phytophthora*	
Bio Vam	*T, harzianum, T, koningii A. glovirormis, A. chrococcum, A. vinelandii, B, subtillis, P. alcaligenes* and *P, putida*	Growth promotion and induces resistance	USA and Canada- T&J Enterprises
Biozera	*T. harzianum* and *B. subtilis*	Growth promoter	Bolivia - Proinpa
Compete Plus	*B. amyloliquefaciens*; *B. pumilus*; *B. subtilis*; *B. licheniformis*; *A. chroococcum* and *T. harzianum*	Soilborne pathogens, growth promotion, and alleviate abiotic stress	EU and UK - Plant Health Cure
PHC Biopak-F	*S. griseoviridis, B. pumilus, B. azotofixans, B. licheniformis, B. megaterium, B. polymyxa* and *B. subtilis*	Soilborne pathogens	USA and Mexico - Plant Health Care
Shocker	*T. harzianum* CPQBA 040-11DRM09 and *B. amyloliquefaciens* CPQBA 040-	*R. solani; S. sclerotiorum.*	Brazil - Agrivalle

	11DRM01 and CPQBA 040-11DRM04		
Subtitricho	*Trichoderma* and bacterium	*Botrytis, Alternaria, Fusarium, Rhizoctonia*	USA and Mexico - Biotecnologia Agroindustrial
TNC Tricorr	*T. hamatum, T. harzianum, T. koningii, T. longibrachiatum, T. reesei, B. subtilis, B. myloliquefaciens* and *B. licheniformis*	*Botrytis, Pythium; Fusarium*.	UK - Nutrient Company
Tricobal	*T. harzianum, T. koningiopsis, B. subtilis* and *B. amyloliquefaciens*	Growth promoter.	Bolivia - Fundación Proinpa
Triconova	*T. harzianum, T. koningiopsis* and *B. subtilis*	*F. oxysporum; B. cinerea.*	Peru - Novagri
Biofit Rut	*B. subtilis, P. billai, P. lilacinus, T. harzianum* and *A. brasilense*	Phosphate solubilization and nitrogen fixation.	Colombia, Mexico and USA - Soluciones Microbianas
Micosat F	*T. harzianum* TH01, *P. chlamydosporia* PC50, *A.*	Soilborne pathogens, growth promotion and induces resistance	Italy and Netherland - CCS AOSTA Quart

	radiobacter AR39, *B. subtilis* BA41, *Streptomyces* SB14		
Ayush	*T. viride, P. fluorescens* and micorryzes	Sigatoka, panama disease, nematodes, soilborne pathogens and rust	India - Bio Sciences
Bactiva	*T. harzianum, T. reesei, T. viride, G. virens, B. polymyxa, B. subtilis, B. megaterium* and *P. fluorescens*	*Pythium, Fusarium, Phytophthora, Rhizoctonia* and *Verticillium.*	USA, EU - Tecnologias Naturales
Biocult Mycorrhiza	*T. harzianum, G. mosseae, G. intraradices, G. etunicatum* and *S. dipurpurescens*	Biostimulant	Zambia, Malawi, Namibia and Zimbabwe - Biocult, NuLandis
BioplantgUard	*Trichoderma* spp. and micorrizal fungi	*Fusarium, Verticillium, Sclerotinia, Botrytis, Pythium; Rhizoctonia*.	Italy - SAIPAN
Condor	*T. atroviride*; *Glomus* spp. and rhizosphere bacteria	*Alternaria, Armillaria, Botrytis, Colletotrichum, Fusarium, Phytophthora, Pyrenochaeta,*	UK - Italpollina

		Pythium, Rhizoctonia, Sclerotinia, Xanthomonas.	
Micover	*T. harzianum, Glomus* spp., *Pseudomonas* spp.	*Phytophthora, Pythium, R. solani, Phoma, Sclerotinia, Verticillium, A. mellea*	Italy - Agrifutur
Nutri-Life Platform	*Trichoderma*	Growth promotion, nutrient solubilization and nitrogen fixation.	Australia and South Africa - Nutri-Tech Solutions
Team Horto	*T. atroviride* MUCL45632; *G. mosseae*, *G. intraradices* and rhizosphere bacteria	Improves plant development and increases yield, fruit quality and crop resistance to climate constraints	Italy - Italpollina
Tifi	*T. atroviride* MUCL45632, *Glomus* spp., rhizosphere bacteria	*Armillaria, Rhizoctonia, Sclerotinia, Fusarium, Phytophthora* and *Botrytis*.	France - Italpollina
TNC Micorr	*Trichoderma, Bacillus*, endo- and ectomycorrhizal fungi	Growth promotion and resistance induction	UK - The Nutrient Company

Adapted from Bettiol et al. [2019].

Table 5. Some products formulated with a mixture of active ingredients registered in Brazil as biofungicides, bioinsecticides and bionematicides.

Product/Company	**Active Ingredient**	**Biological target**
Agtecmmon - Massen	*Bacillus amyloliquefaciens* CPQBA 040-11DRM 01 and CPQBA 040-11DRM 04	*Colletotrichum lindemuthianum; C. truncatum; Corynespora cassiicola; Phaeosphaeria maydis; Ramularia areola.*
Ataplan and Aratel - FMC	*Bacillus subtilis* RTI477 and *Bacillus velezensis* RTI301	*Colletotrichum dematium*; *Pythium* spp., *Rhizoctonia*, *Fusarium oxysporum* f. sp. *vasinfectum*
BF20.001 -Ballagro	*Trichoderma harzianum* URM 8119; *Trichoderma asperellum* URM 8120 and *B. amyloliquefaciens* CCT 7901	*R. solani*; *Sclerotinia sclerotiorum*; *C. lindemuthianum.*
Bombardeiro and Lastro - Total	*B. subtilis* CCTB04; *B. velezensis* CCTB09, and *Bacillus pumilus* CCTB05	*Septoria glycines.*
Native - Agrivalle	*B. amyloliquefaciens* CPQBA 040-11DRM 01 and CPQBA 040-11DRM 04, *T. harzianum* CPQBA 040-11DRM 09	*R. solani; S. sclerotiorum.*
Mbyo Tric and Rnbio Tric – MB Enzymes	*T. harzianum* IBLF1278 and IBLF1282, *Trichoderma viride* IBLF1275 and IBLF1276	*R. solani; F. oxysporum.*

Ourotricx – Ballagro	*T. harzianum* URM 8119, *T. asperellum* URM 8120, *B. amyloliquefaciens* CCT 7901	*R. solani; S. sclerotiorum; C. lindemuthianum.*
Pardella - Ballagro	*T. harzianum* URM 8119, *T. asperellum* URM 8120, and *B. amyloliquefaciens* CCT 7901	*C. lindemuthianum; S. sclerotiorum; R. solani.*
Provilar - FMC	*B. velezensis* RTI301 and *B. subtilis* RTI477	*S. sclerotiorum*
Shocker; Peak Goolan, Biovenci - Agrivalle	*B. amyloliquefaciens* CPQBA 040-11DRM 01 and CPQBA 040-11DRM 04, and *T. harzianum* CPQBA 040-11DRM 09	*R. solani; Fusarium solani*; *S. sclerotiorum.*
Tanus – Biota Innovations	*T. harzianum* URM 8119; *T. asperellum* URM 8120 and *B. amyloliquefaciens* CCT 7901	*R. solani*; *S. sclerotiorum; C. lindemuthianum*.
Biotricho - Biomip	*T. harzianum* IBLF1278; *T. harzianum* IBLF 1282; *T. viride* IBLF1275 and *T. viride* IBLF1276	*R. solani*, *F. oxysporum.*
BTP 010-19, BTP 010-19A - Total	*B. subtilis* CNPSo2720; *B. velezensis* CNPSo 3602 and *B. pumilus* CNPSo3203	*S. glycines.*
Twixx; Amosbio Goplan; Bio Braza – Agrivalle	*B. amyloliquefaciens* CPQBA 040-11DRM 01 and CPQBA 040-11DRM 04	*C.* spp.*; Phaeosphaeria maydis; R. areola.*

Torpeno; Bionativos; Bellator and Trichosmart - Massen	*B. amyloliquefaciens* CPQBA 040-11DRM 01 and CPQBA 040-11DRM 04, and *T. harzianum* CPQBA 040-11DRM 09	*R. solani*; *S. sclerotiorum.*
Tricozak - Cooperativa Mista de Des. Agronegócio	*T. harzianum* URM 8119*; T. asperellum* URM 8120 and *B. amyloliquefaciens* CCT 7901	*R. solani*; *S. sclerotiorum*; *C. lindemuthianum.*
Trichoagro – JCO	*T. harzianum* IBLF1278 and IBLF1282; *T. viride* IBLF1275 and IBLF1276	*F. oxysporum; R. solani.*
Trichoderma Bom Futuro – Bom Futuro	*T. harzianum* IBLF1278 and IBLF1282; *T. viride* IBLF1275 and IBLF1276	*F. oxysporum; R. solani.*
TrichofourT – Biossíntese	*T. harzianum* IBLF1278 and IBLF1282; *T. viride* IBLF1275 and IBLF1276	*F. oxysporum; R. solani.*
TrikoSoil – Solubio	*T. harzianum* IBLF1278 and IBLF1282; *T. viride* IBLF1275 and IBLF1276	*F. oxysporum; R. solani.*
DuoControl - Simbiose	*Beauveria bassiana* IBCB 66 and *Metarhizium anisopliae* IBCB 425	*Deois flavopicta*; *Euschistus heros.*
Assertive and Duotrix – Agroecológica	*M. anisopliae* IBCB 425 and *B. bassiana* IBCB 66	*D. flavopicta*; *E. heros.*
Biokato, BTP 016-19 – Total	*Pseudomonas fluorescens* CCTB03 and *Pseudomonas chlororaphis* CCTB19	*B. tabaci; D. maidis; E. heros.*

BioMatch - JCO	*M. anisopliae* IBCB 425 and *B. bassiana*, IBCB 66	*D. flavopicta*; *E. heros.*
BioOlimpo – Biotrop	*B. bassiana* IBCB 66 and *M. anisopliae* IBCB 425	*D. flavopicta*; *E. heros.*
BioSCAP and BioSCAP – Vital Brasil Chemical	*B. bassiana* IBCB 66 and *M. anisopliae* IBCB 425	*D. flavopicta*; *E. heros.*
BI2003/16 - Ballagro	*B. bassiana* IBCB 66 and *M. anisopliae* IBCB 425	*Bemisia tabaci*; *D. flavopicta*; *E. heros.*
BMS Max - Prophyto	*M. anisopliae* IBCB 425 and *B. assiana* IBCB 66	*D. flavopicta*; *E. heros.*
Bometal - Ballagro	*B. bassiana* IBCB 66 and *M. anisopliae* IBCB 425	*B. tabaci*; *H. hampei*; *D. flavopicta*; *E. heros*; *Sphenophorus levis*; *Tetranychus*; *Frankliniella*
Bove-Meta Bom Futuro – Bom Futuro	*B. bassiana* IBCB 66 *and M. anisopliae* IBCB 425	*D. flavopicta; E. heros.*
BTP 078-20 – Total	*B. bassiana* IBCB 66 and *M. anisopliae* IBCB 425	*D. flavopicta*; *E. heros.*
Celtic – Ballagro	*B. bassiana* BALL 6-2, *Isaria javanica* URM 7662	*Unaspis citri; Diaphorina citri; B. tabaci* raça B.

Dobbel – Agroecológica	*M. anisopliae* IBCB 425 and *B. bassiana* IBCB 66	*D. flavopicta*; *E. heros*.
Mesopel Mix – Dillon	*B. bassiana* IBCB 66 and *M. anisopliae* IBCB 425	*D. flavopicta; E. heros.*
Squadra - Ecosolução	*B. bassiana* IBCB 425 and *M. anisopliae* IBCB 66	*D. flavopicta; E. heros.*
Surtivo soja – AgBTech	*Baculovirus Chrysodeixis includens* and *Baculovirus Helicoverpa armigera*	*Helicoverpa armigera; Chrysodeixis includens.*
Takotrop – Total	*P. fluorescens* CCTB03 and *P. chlororaphis* CCTB19	*E. heros*; *D. maidis*; *B. tabaci*
Tbio – Biomip	*B. bassiana* IBCB 66 and *M. anisopliae* IBCB 425	*D. flavopicta*; *E. heros*.
Bio Tramo – Agrivalle	*B. subtilis* ATCC 6051, *B. licheniformis* ATCC 12713 and *P. lilacinus* CPQBA 040-11 DRM 10	*M. incognita*; *P. brachyurus*.
Nemabac and Bamytis – Biota Innovations	*B. amyloliquefaciens* SVG 00027-B and SVG 00028-B; *B. thuringiensis* SVG 00029-B and *B. subtilis* SVG 00030-B	*M. incognita.*
Nimaxxa, Unnat – Chr. Hansen	*Bacillus paralicheniformis* CH2970 and CH0273 and *B. subtilis* CH4000	*M. incognita; H. glycines; P. brachyurus.*
Profix, Volga – Agrivalle	*B. subtilis* ATCC 6051, *B. licheniformis* ATCC 12713 and *P. lilacinus* CPQBA 040-11 DRM 10	*M. incognita*; *P. brachyurus*.

Presence, FortMax, Quartzo and Surface – FMC	*B. subtilis* FMCH002 (DSM32155) and *B. licheniformis* FMCH001(DSM32154)	*P. brachyurus*, *Pratylenchus zeae*; *Meloidogyne* spp.; *Radopholus similis*; *Heterodera glycines*.
ACC Max; Biomagno; Protege; Quorum - Total	*B. amyloliquefaciens* CNPSo3202, *B. velezensis* CNPSo3602 and *B. thuringiensis* CNPSo3915	*R. solani; P. brachyurus.*

Data obtained of official site of Agriculture Minister (https://agrofit.agricultura.gov.br/) accessed in 06/02/2023.

Artigo 2: FORMULATIONS CONTAINING MICROBIAL CONSORTIUM TO PROTECT BEAN PLANTS AGAINST PESTS AND DISEASES

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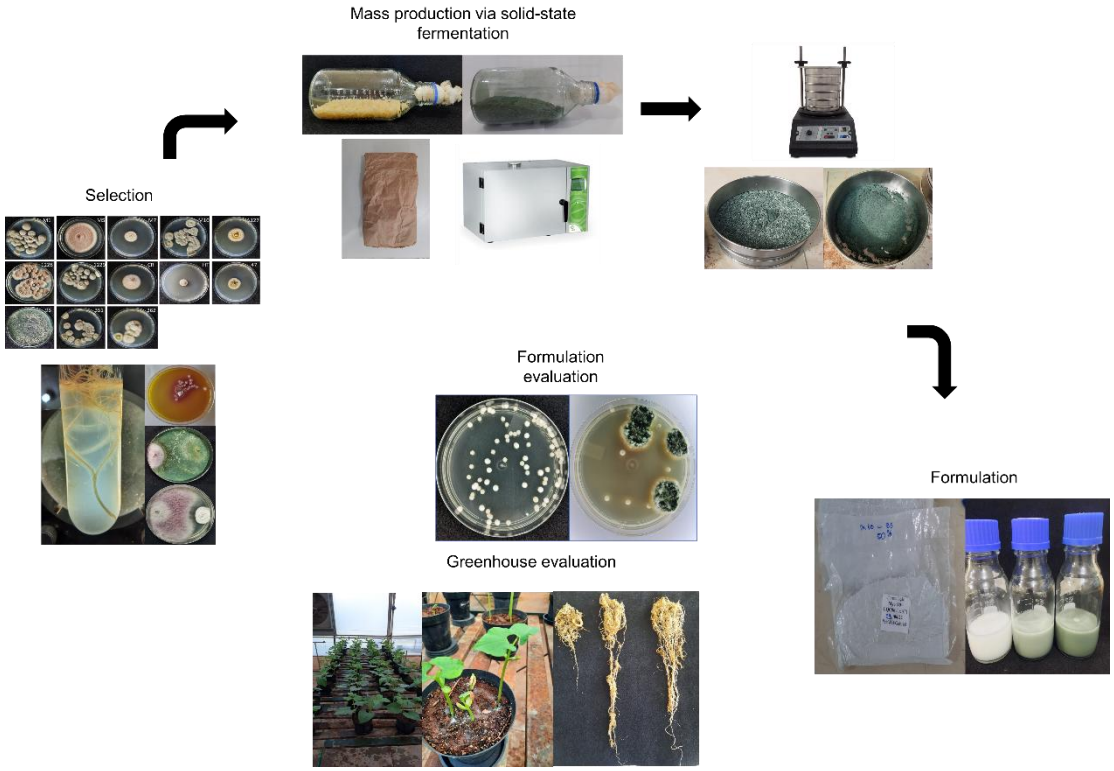
Graphical abstract



ABSTRACT

The use of biological control agents to manage diseases in agriculture has been receiving increasing attention. This has posed a significant challenge in the biological products industry: the development of new formulations that enhance the efficacy of active agents. In this study, the aim was to develop wettable powder (WP) and emulsifiable concentrate (EC) formulations containing *Trichoderma asperelloides* and entomopathogenic fungi, both mixed and separately, and evaluate their efficacy in controlling Fusarium wilt in common beans caused by Fusarium oxysporum f. sp. phaseoli (FOP), as well as their potential as growth promoters in bean plants. To achieve this, 11 isolates of entomopathogenic fungi from different genera were evaluated for their compatibility with *T. asperelloides* (LQC-96) and their in vitro antagonism against FOP. *Beauveria bassiana* (M10) was selected to be combined with *T. asperelloides* (LQC-96), and the microorganisms were formulated both together and separately. The formulations were then tested for storage stability and their efficacy in controlling *Fusarium* wilt and promoting growth in bean plants, applied both mixed and separately. The formulations-maintained conidia viability for up to eight months of storage. Additionally, the formulations were effective in controlling *Fusarium* wilt in common beans. There was also a significant increase in root length, volume, and fresh and dry masses of the bean plants compared to the control infested with *Fusarium* (FOP). The wettable powder formulation containing the mixture of *Trichoderma* and *Beauveria* showed higher control efficacy.

Keywords: Biological control, biofungicides, wettable powder, emulsion concentrated,

1.INTRODUCTION

Chemical pesticides have been widely used for insect and disease management for decades, significantly impacting food production (SHARMA, et al., 2019). However, the recurring adverse effects on human health, the environment, and the selection of resistant pests have raised questions about the widespread use of pesticides. Among the alternatives to reduce reliance on chemical control, biological control is gaining importance as another ally in integrated pest and disease management programs (LACEY et al., 2015).

The screening of promising strains and further development of new biological products have been gaining significant momentum. It is estimated that the global market for biological products will grow at a Compound Annual Growth Rate (CAGR) of 13.8%, jumping from an estimated value of $14.6 billions in 2023 to $27.9 billions in 2028 (MARKETS; MARKETS, 2024). Among the products registered in Brazil, bioinsecticides based on *Beauveria bassiana* (*stricto sensu*), *Metarhizium anisopliae* (ss) and *Bacillus thuringiensis* have the highest number of registered products, with 103, 100, and 54, respectively (AGROFIT, 2024). Additionally, 18 products are a mix of *B. bassiana* and *M. anisopliae*. For biofungicides, the genera *Bacillus* sp. and *Trichoderma* are the most important, with 149 and 75 registered products, respectively. No formulations have *Trichoderma* isolates mixed with other microrganisms

The genus *Trichoderma* (Ascomycota: Hypocreales) is one of the most studied for biological control of plant diseases, widely employed as an antagonist to various phytopathogens, especially those inhabiting the soil (Bettiol et al., 2019). *Trichoderma* are free-living, cosmopolitan, non-pathogenic, and commonly found associated with the organic fraction of the soil and plant rhizospheres (VERMA et al., 2007). Moreover, fungi within this genus possess a broad arsenal of action mechanisms, such as antibiosis, production of hydrolytic enzymes, competition, and mycoparasitism. They can also induce systemic resistance and promote plant growth by increasing hormone production and nutrient availability (HYNES, 2006; MONTE et al., 2019).

The entomopathogenic fungus *Beauveria bassiana* (Ascomycota: Cordycipitaceae) is one of the most important biological agents employed in the control of agricultural pests. This genus is also reported as growth promoters, endophytes, and resistance inducers (SIQUEIRA, 2020; DEB et al., 2020; BARRA-BUCAREI et al., 2020; CACHAPA, 2021). B. bassiana has been reported to control various fungal diseases such as wilts, leaf spots, blights, root rot, as well as diseases caused by bacteria and viruses (DEB et al., 2020). The potential for the multiple uses of this microorganism, both for arhtropod management and disease control, opens up an

important avenue for biological control and highlights the importance of new studies exploring the multifunctionality of fungal entomopathogens.

Solid-state fermentation is the most commonly used method for mass production of biocontrol fungi due to its production ease, higher tolerance of conidia to abiotic factors, process simplicity, high yields, and low energy usage (LU et al., 1998; BRAMORSKI, 1997). For formulation, it is initially necessary to produce a large quantity of propagules (mycelium, aerial conidia and, in some cases, chlamydospores). Subsequently, a diluent (solid or liquid) and adjuvants are added to these propagules, aiming to provide protection, dispersion, adhesion, and improvement in cell multiplication and survival under adverse conditions. Therefore, formulation is essential for the development of new products based on microbial agents.

Brazil is one of the largest global producers of beans (*Phaseolus vulgaris* L.), with a planted area of 843 thousand hectares n Jan/2024 (first crop) and an estimated yield of 1,116 kg ha^{-1} (CONAB, 2024). Bean cultivation holds significant economic and social importance. However, despite Brazil being one of the world's leading bean producers, the yield remains low, with the occurrence of pests being a major contributing factor. One disease of significant importance to the crop is *Fusarium* rot, caused by a complex of *Fusarium* species, including the fungus *Fusarium oxysporum* f. sp. *phaseoli*. This pathogen is known for the difficulty in its management due to its ability to survive in crop residues, alternate hosts, and soil through the production of resistance structures, known as chlamydospores. Managing this disease is also considered challenging due to the low efficiency of recommended chemical fungicides.

The present study aims to select entomopathogenic fungi for the biological control of *Fusarium oxysporum* f. sp. *phaseoli* (FOP) and develop formulations based on Trichoderma and an entomopathogenic fungus, both in combination and separately. The goal is to evaluate the efficiency of the studied formulations in controlling bean wilt caused by FOP and promoting the growth of bean plants and controlling whitefly.

2.MATERIALS AND METHODS

The study was conducted at the "Raquel Ghini" Environmental Microbiology Laboratory of Embrapa Meio Ambiente (22°43'36" S and 47°00'59" W), at Jaguariúna, State of São Paulo. The climate in the region is classified as humid subtropical (Cfa according to the Köppen classification), characterized by hot and rainy summers and cold and dry winters.

2.1. Microorganisms

In the study, the isolate CMAA 1584 of *Trichoderma asperelloides* (BRM 065723, GenBank accession ON542481) was used. This isolate was obtained from soil in the experimental area of Embrapa Meio Ambiente and deposited in its collection, known as Collection of Microorganisms of Agricultural and Environmental Importance. The microrganisms used in the study are described in Table 1.

Fusarium oxysporum f. sp. *phaseoli* (CML 144), isolated from the stem of *Phaseolus vulgaris* at Lavras (21º13´44.9S and 44º58´50.8W), Minas Gerais state, was provided by the Lavras Mycology Collection. For preservation of isolates, sporulated colonies were cut into approximately 5 mm fragments, placed in cryotubes containing 1.5 mL of sterilized 20% (v/v) glycerol solution (Dinâmica®, SP, Brazil), and stored at -20 ºC. The isolates were reactivated on Petri dishes containing potato dextrose agar (PDA; Acumedia Manufacturers®, Michigan, USA) and maintained for 15 days at 28 ± 2 ºC in a BOD incubator.

2.2. Mycelial Growth Rate Index (MGRI) and Mycelial Growth Rate Potential (%MGRI)

The methodology applied in this experiment was based on Jackisch - Matsuura and Menezes (1999) for determining the mycelial growth and growth rate of isolates. Discs with a diameter of 5 mm containing fungal structures with 15 days of age were transferred to Petri dishes containing BDA medium. The plates were sealed and maintained at a temperature of 28 ± 2 °C under a 12-hour photoperiod. The experiment was conducted in a completely randomized design. Radial mycelial growth was quantified by measuring (mm) the diameter, previously marked on the outer part of the Petri dish bottom with the aid of a millimeter ruler, at three-time intervals: 24, 48, and 72 hours. The results were used to calculate the MGRI (Mycelial Growth Rate Index) according to a formula modified from Oliveira (1991), and the growth expressed in cm per 24 hours: MGRI = ((D24h – 0) + (D48h – D24h) + (D72h – D48h)) / 3. Where: MGRI = Mycelial Growth Rate Index, D24h = Average diameter at 24 hours of incubation, D48h = Average diameter at 48 hours of incubation, and D72h = Average diameter at 72 hours of incubation. The individual percentages of MGRI (%MGRI) for the evaluated isolates were determined, taking the highest average mycelial growth rate value as 100%.

Table 1. Isolates of entomopathogenic fungi used in the selection for the control of phytopathogens.

Isolats	Work code	Scientific name	Code	Origin Collection
CMAA1807	M1	*Beauveria bassiana*	BRM 047250	CMAA
MYCO-05	M5	*Purpureocillium lilacinum*	BRM065722	CMAA
CMAA1810	M7	*Beauveria caledonica*	BRM 061147	CMAA
CMAA1813	M10	*Beauveria bassiana*	BRM 061405	CMAA
CG1127	1127	*Metarhizium robertsii*	BRM 4683	CFI
CG1228	1228	*Cordyceps javanica*	BRM 14526	CFI
CMAA1655	1229	*Beauveria bassiana*	BRM 14527	CMAA
CMAA1284	CR	*Clonostachys rosea*	BRM 065724	CMAA
CG511	HT	*Hirsutella thompsonii*	BRM 2806	CFI
CG47	47	*Metarhizium anisopliae*	BRM 2136	CFI
IP351	351	*Beauveria bassiana*	-	-
CG632	632	*Metarhizium robertsii*	BRM 2813	CFI

∗ CMAA=Coleção de Microrganismos de Importância Agrícola e Ambiental-Embrapa Meio Ambiente. CFI= Coleção de Fungos de Invertebrados-Embrapa Cenargen.

2.3. Detection of Chitin-Degrading Microorganisms

Colloidal chitin was prepared from commercial shrimp shell chitin (Sigma, Saint Louis, USA) using the method of Roberts and Selitrennikoff (1988) with some modifications, and supplemented in the chitinase assay medium as the sole carbon source. The acid hydrolysis of chitin was carried out with hydrochloric acid (HCl) by constant stirring using a magnetic stirrer at ± 4 °C (refrigerator) overnight, followed by extraction of colloidal chitin in 2,000 ml of neutralization with 95% ethanol kept at 26 °C overnight. Subsequently, it was centrifuged at 3,000 rpm for 20 minutes at 4 °C. The sediment was washed with sterile distilled water by

centrifugation at 3,000 rpm for 5 minutes at 4 °C until the alcohol smell was completely removed. The obtained colloidal chitin had a soft pasty consistency with 90-95% water content and was stored at 4 °C for later use.

The medium for chitinase detection was prepared by adding 0.3 g $MgSO_4.7H_2O$, 3.0 g $(NH_4)_2SO_4$, 2,0 g KH_2PO_4, 1.0 g monohydrated citric acid, 15 g agar, 200 µl Tween 80, 4.5 g moist colloidal chitin, 0.15 g bromocresol purple, and 1,000 mL distilled water. The pH was adjusted to 4.7. The medium was autoclaved at 121 °C for 15 minutes and then poured into Petri dishes. Discs of approximately five mm in diameter from the colonies of the isolates to be tested for chitinase activity (Table 1) were transferred to Petri dishes containing the chitinase detection medium and incubated at 25 ± 2 °C for seven days. They were observed for the formation of colored zones around the colony. The chitin degradation halo was measured with a caliper, and the results were expressed in mm.

2.4. Phosphate Solubilization

To assess the potential of the *Trichoderma* isolate and entomopathogenic isolates in phosphate solubilization, a five mm disc containing fungal structures was placed in the center of a Petri dish containing NBRIP (National Botanical Research Institute's Phosphate) medium, which includes per liter: 10 g glucose; 5g $Ca_3(PO_4)_2$; 5g $MgCl_2.6H_2O$; 0.25 g $MgSO_4.7H_2O$; 0.2 g KCl; 0.1 g $(NH_4)_2\ SO_4$; 15 g agar, and pH 7.0 (Nautiyal, 1999), with 0.1% Triton X100 (Synth®, Diadema, SP, Brazil). The plates were incubated at 25 ± 2 °C for 5 days with a 12:12 h photoperiod. The formation of a clear hydrolysis halo around the colonies was evaluated, confirming the ability to solubilize calcium phosphate.

The solubilization of inorganic phosphate (P) was also verified in NBRIP liquid medium, prepared as previously described without the addition of agar. In the medium, 50 mL of K_2HPO_4 (10%) and 100 mL of $CaCl_2$ (10%) were added to form an insoluble calcium phosphate precipitate ($CaHPO_4$). For inoculum production, 7-day-old sporulated cultures of each strain were washed with 10 mL of a sterile NaCl (0.085%) saline solution containing 0.04% polyoxyethylene sorbitan monooleate (Tween® 80, Synth, SP, Brazil) and calibrated using a hemocytometer (enhanced Neubauer chamber, 400x magnification) under a microscope (DM 500, Leica Microsystems GmbH®, Germany) to provide a final inoculum size of 5×10^6 conidia mL^{-1} in the medium. These liquid cultures were then incubated at 28 ± 1°C on an orbital rotary shaker (TE1401, Tecnal®, Piracicaba, SP, Brazil) at 180 rpm for 5 days with a 12:12-hour photoperiod. The amount of calcium phosphate in the medium before inoculation of

Trichoderma strains was approximately 150 µg mL^{-1}. One-milliliter aliquots were taken on the 5th day and centrifuged at 7,000 rpm and 22°C for 5 minutes to determine the concentration of soluble phosphorus, according to the colorimetric method described by Murphy and Riley (1962). The concentration of solubilized P in the supernatant was calibrated based on a standard curve of $CaHPO_4$ (SigmaAldrich®, St. Louis, MO, USA) at concentrations of 0.5, 1.0, 2.0, 2.5, and 5.0 mg mL^{-1}. The experiments were conducted with four biological replicates for each fungal strain. The untreated control group (blank) was performed without the presence of microorganisms, and the values obtained were subtracted from those obtained in the presence of fungal inoculum as a way to normalize absorbance readings.

2.5. In vitro antagonism assessment: Co-cultivation

The ability of *T. asperelloides* CMAA1584 and other isolates to antagonize *F. oxysporum* f. sp. *phaseoli* (FOP) was assessed through plate confrontation on Petri dishes containing 20 mL of PDA medium. For this purpose, the organisms were grown on Petri dishes for 7 days at 25 ± 2ºC. Subsequently, the isolates were confronted with FOP, with a PDA disk containing pathogen structures placed at one end of the plate and a disk containing one of the potential antagonist isolates at the other end. The test was conducted by either transferring the pathogen and biological control agent simultaneously or transferring the pathogen three days after the transfer of the biological control agent. Evaluations were performed daily from the second day by measuring the growth of both fungi. The plates were incubated as previously mentioned and assessed until the growth of FOP in the control treatment reached the other end of the plate.

2.6. Metabolite Production

The mass production of the selected organisms was carried out through solid-state fermentation, using Type 1 parboiled rice (Broto Legal Alimentos S.A., SP-Brazil) as a substrate. For this purpose, 150 g of parboiled rice, previously moistened with 187.5 mL of distilled water for 50 minutes, was placed in 1000 mL transparent Schott glass bottles and autoclaved at 121 °C and 1 atm pressure for 20 minutes. For the inoculum, the fungi were grown on Petri dishes containing PDA medium, and the plates were kept for ten days at 25 ± 2 °C. Conidial suspensions of the fungi were obtained from plates containing cultures of sporulated fungi; the spores were washed with 10 ml of 0.85% NaCl saline solution plus 0.04% Tween 80 (Tween

80, Synth®, Diadema, SP, Brazil), followed by scraping the conidia with a Drigalski loop. Subsequently, the substrates were inoculated with a conidial suspension calibrated to a final concentration of 1 x 10^6 conidia g^{-1} of rice and incubated at room temperature at 28 ± 2 °C for seven days.

After the incubation period, conidia were extracted to prepare the formulations. For this, the colonized rice was transferred to kraft paper bags (24 x 12 x 07 cm), sealed, and placed in a climatic chamber (Nova Ética®, model: 410/3ND) for three days for drying the colonized rice. Only air circulation was activated in the chamber, and the rice was dried at room temperature. The moisture content of the colonized rice grains after drying was also determined using a moisture analyzer ID-50 (Marte Científica®, São Paulo, SP, Brazil), with the moisture content determined from six randomly collected samples.

After drying, the rice was transferred to a set of sieves with 16 and 32 mesh sizes and placed on a sieve shaker (Bertel®, Caieiras, SP) for 60 minutes at vibration intensity level 10. After this period, the isolated spores collected at the bottom of the sieve set were transferred to Petri dishes and stored in desiccators with silica gel, and refrigerated at 4ºC until formulation.

The concentration of conidia present in the spore powder was determined using a Neubauer chamber. For this purpose, 0.1 g of spore powder from each isolate was added to a 250 mL Erlenmeyer flask containing 99.9 g of autoclaved NaCl (0.85%) saline solution supplemented with Tween 80 (0.04%). This dilution corresponds to a 10^{-3} dilution. The Erlenmeyer flasks with the suspension were placed on an orbital shaker for 60 minutes at 120 rpm, followed by immersion in an ultrasonic bath (LAB-LÍDER®, Ribeirão Preto, SP, Brazil) for 5 minutes. One mL of this suspension was transferred to nine mL of saline solution with Tween, resulting in a 10^{-4} dilution. This 10^{-4} suspension was pipetted onto a Neubauer chamber with a mirrored bottom (Global Optics®) and counted under a microscope (OLYMPUS® CX 43) at 200x magnification, counting both chamber fields, and repeating the count twice.

The viable spore count was also determined using the colony-forming unit (CFU) methodology. Dilutions were carried out as described previously, obtaining dilutions of 10^{-3}. Subsequent dilutions were performed in test tubes by adding one mL of the previous dilution to nine mL of autoclaved NaCl (0.85%) saline solution with Tween 80 (0.04%). In the end, aliquots of 0.1 mL from the 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} dilutions were plated on PDA + Triton X100 0.04% agar medium (SYNTH®, Diadema, SP, Brazil) and incubated at 28 ± 2 °C with a 12:12 h light-dark cycle in a BOD-type incubator. Colony counts were performed after 48h and 72h of incubation. Dilutions with colony counts ranging from 30 to 300 were used for calculations.

2.7. Endophytisms and colonization of bean plants

Bean seeds (*Phaseolus vulgaris* L.) cv. BRS Estilo (SEEDS ORIENT®, Patos de Minas, MG, Brazil) were superficially disinfected by sequential agitation in 70% ethanol and 2% sodium hypochlorite solutions for 1 minute each, thoroughly washed three times in sterile distilled water, and then dried in a laminar flow chamber for one hour. The superficially sterilized bean seeds were immersed in 5 mL suspensions containing 1×10^8 spores/mL of the suspension of each tested isolate (Table 1), which were cultivated as described earlier. The seeds were then dried in a laminar flow chamber in open Petri dishes for 24 hours. The seeds were aseptically transferred to Petri dishes containing 1.5% water-agar and incubated at 25 ± 2 °C in the dark for germination. After three days, the seedlings were aseptically transferred to tubes (4.5 cm in diameter, 24 cm in length) containing Phytagel-water (SIGMA-ALDRICH, St. Louis, MO, USA), at 1.2% w/v. The tubes were kept in a growth chamber at 28 ± 2 °C, with a 12:12 hour photoperiod. After seven days of incubation, root system colonization was considered positive when a cloudy zone around the roots was clearly seen. Direct isolation from the root system and aerial parts of the bean plants was also performed. For this, 1 cm fragments of stems and roots were collected from the plants, superficially disinfected in 70% alcohol (for 2 minutes), 2.5% hypochlorite (for 2 minutes), and washed three times in autoclaved distilled water. Subsequently, the fragments were cut into smaller pieces and transferred to PDA medium.

Three tubes were used for each isolate. For direct isolation, each tube gave rise to six plates, three plates with root fragments and three plates with stem fragments. Each plate contained five fragments. Bean seeds treated with autoclaved distilled water were used as the control. The assay was repeated, on a different date, to confirm the results.

2.8. Compatibility between biocontrol agents

Compatibility tests between *Trichoderma asperelloides* and entomopathogenic isolates were conducted on Petri dishes containing BDA medium using the co-culture method, aiming to identify a compatible combination of entomopathogens with T. asperelloides for consortium development. The interaction between colonies was visually assessed following the method described by Mohammad et al. (2011). The plates were incubated at 28 ± 2 °C. The experiments

were arranged in a completely randomized design with three replications. The assay was repeated to confirm the results.

2.9. Mass production through solid-state fermentation

The mass production of the selected organisms was carried out through solid-state fermentation, using Type 1 parboiled rice (Broto Legal Alimentos S.A., SP-Brazil) as a substrate. For this purpose, 150 g of parboiled rice, previously moistened with 187.5 mL of distilled water for 50 minutes, was placed in 1000 mL transparent Schott glass bottles and autoclaved at 121 °C and 1 atm pressure for 20 minutes. Subsequently, the substrates were inoculated with a conidial suspension calibrated to a final concentration of 1 x 10^6 conidia g^{-1} of rice and incubated at room temperature for seven days. For the inoculum, the fungi were grown on Petri dishes containing BDA medium, and the plates were kept for ten days at 25 ± 2°C. Conidial suspensions of the fungi were obtained from plates containing cultures of sporulated fungi; the spores were washed with 10 ml of 0.85% NaCl saline solution plus 0.04% Tween 80 (Tween 80, Synth®, Diadema, SP, Brazil), followed by scraping the conidia with a Drigalski loop.

After the incubation period, conidia were extracted to prepare the formulations. For this, the colonized rice was transferred to kraft paper bags (24 x 12 x 07 cm), sealed, and placed in a climatic chamber (NOVA ÉTICA®, model: 410/3ND) for three days for drying the colonized rice. Only air circulation was activated in the chamber, and the rice was dried at room temperature. The moisture content of the colonized rice grains after drying was also determined using a moisture analyzer ID-50 (Marte Científica®, São Paulo, SP, Brazil), with the moisture content determined from six randomly collected samples.

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mL of this suspension was transferred to nine mL of saline solution with Tween, resulting in a 10^{-4} dilution. This 10^{-4} suspension was pipetted onto a Neubauer chamber with a mirrored bottom (Global Optics®) and counted under a microscope (OLYMPUS® CX 43) at 200x magnification, counting both chamber fields, and repeating the count twice.

The viable spore count was also determined using the colony-forming unit (CFU) methodology. Dilutions were carried out as described previously, obtaining dilutions of 10-3. Subsequent dilutions were performed in test tubes by adding one mL of the previous dilution to nine mL of autoclaved NaCl (0.85%) saline solution with Tween 80 (0.04%). In the end, aliquots of 0.1 mL from the 10-6, 10-7, 10-8, and 10-9 dilutions were plated on BDA + Triton X100 0.04% agar medium (SYNTH®, Diadema, SP, Brazil) and incubated at 28 ± 2 °C with a 12:12 h light-dark cycle in a BOD-type incubator. Colony counts were performed after 48h and 72h of incubation. Dilutions with colony counts ranging from 30 to 300 were used for calculations.

2.10. Formulation of biocontrol agents

Based on the results of the assays described in the previous sections, the entomopathogenic isolate that was part of the consortium, along with the *Trichoderma* isolate LQC 96, was selected. The selected microorganism was *Beauveria bassiana* isolate MYCO10, as it demonstrated greater efficiency in in vitro tests. These isolates were separately produced via solid-state fermentation, using rice grains as a substrate, and formulated separately and together in wettable powder and concentrated emulsion formulations. For the formulations with the mixture, the isolates were individually formulated and then mixed in equal proportions before storage.

Wettable Powder Formulation (WP)

For the wettable powder formulation, neutral talc (CHEMCO®, Hortolândia, SP, Brazil) was used as the carrier (bulk density 1.3-1.38 g mL^{-1} and maximum moisture 0.05%). The formulation included 10% (w/w) of the biodegradable spreader BREAK-THRU® SD 260 (EVONIK®, Essen, Germany), 10% (w/w) of soluble starch (corn starch, Amylodextrin) CS 3400 (ARGO®, Mogi Guaçu, SP, Brazil), and fungal spores to achieve a final concentration of 1 x 10^9 conidia g^{-1} of WP (w/w). Talc was used to bring the formulation to 100% (w/w).

Formulation developed by the authors based on preliminary datarmulation developed by the authors.

Emulsifiable Concentrated Emulsion (EC)

In this formulation, sunflower oil (Lyza®, São Paulo, SP, Brazil) was used as the carrier, along with 2% (w/w) of the dispersant BREAK-THRU® DA 646 (EVONIK®, Essen, Germany), 7% (w/w) of the emulsifier BREAK-THRU® EM V 20 (EVONIK®, Essen, Germany), and fungal spores to achieve a concentration of 1 x 10^{10} conidia mL^{-1} of EC (w/w). Sunflower oil was used to bring the formulation to 100% (w/w). Mixing of the formulation components was done using a manual mixer M150 (Black Decker®, Uberaba, MG, Brazil) and an ultrasonic bath (LAB-LÍDER®, Ribeirão Preto, SP, Brazil) for five minutes. Formulation developed by the authors based on preliminary datarmulation developed by the authors.

Storage stability of the formulations.

The viability of the formulations was assessed at time 0 (immediately after drying) and monthly throughout the entire storage period. For this purpose, 1g samples of the formulated products were collected and added to test tubes containing 9 mL of a saline solution with NaCl (0.85%) + polysorbate 80 at 0.04% (Tween 80, Synth®, Diadema, SP, Brazil), and then serially diluted. Subsequently, aliquots of 0.1 mL from the dilutions were plated on BDA + Triton X100 0.04% agar medium (SYNTH®, Diadema, SP, Brazil) and incubated for 48 to 72 hours at 28 ± 2 °C with a 12:12 h light-dark cycle in a BOD-type incubator.

Additionally, 1g samples of each formulated product were collected in cryopreservation tubes and stored in a refrigerator (4 °C) and at 40 °C. The evaluated samples were destructive, meaning that each sample used one tube containing one gram of the formulated product. The experiment was conducted in a completely randomized design with three replications. The results were expressed in colony-forming units per gram of formulated product (CFU g^{-1}).

2.11. Fusarium Wilt Control in Common Bean Plants

In trials aimed at controlling Fusarium wilt, a mixture of Tropstrato HT Hortaliças® substrate (Mogi Mirim, SP) and soil in a 1:3 (v/v) ratio was used in 0.750 L plastic pots. The soil was collected from the experimental farm area of Embrapa Meio Ambiente.

The soil was collected from the farm area experimental project by Embrapa Meio Ambiente, presenting the following chemical and physical analyzed at 0-20 cm depth: pH em H_2O = 4,3; M.O. = 32,3 g Kg^{-1} ; P = 9,36 mg dm^{-3} ; Ca = 3,09 cmolc dm^{-3} ; Mg = 1,48 cmolc dm^{-3} ; K = 128,55 mg dm^{-3} ; SB = 4,95 cmolc dm^{-3} ; H+Al = 6,10 cmolc dm^{-3} ; t = 4,99 cmolc dm^{-3} ; V% = 44,54. For each kilogram of substrate, 5 g of NPK formula (10, 10, 10) was added. The substrate was infested with FOP chlamydospores produced in talc. The concentration of FOL was adjusted to $1x10^5$ g^{-1} of substrate, except for the absolute control, which was not infested.

To produce FOP chlamydospores in talc, 5 mm diameter discs from FOP colonies, previously grown on PDA medium for seven days at 25 ± 2°C, were transferred to 1,000 mL Erlenmeyer flasks containing 500 mL of BD culture medium (potato-dextrose, KASVI®, Mumbai, India) and maintained at 25 ± 2°C with constant shaking at 150 rpm for seven days. For 1,000 g of neutral talc (CHEMCO®, Hortolândia, SP, Brazil), 500 mL of the inoculum suspension produced in liquid medium was transferred. The mixture was then homogenized to a paste consistency and allowed to dry for 15 days at 21 ± 2°C (DE CAL et al., 1995) in an air-conditioned room. After drying, the number of colony-forming units (CFU) of the inoculum was determined by the serial dilution method, plating dilutions 10^{-5}, 10^{-6}, and 10^{-7} on Nash-Snyder semi-selective culture medium for *Fusarium* spp. (NASH, 1962). The average of dilutions 10^{-5} and 10^{-6} was used to calculate CFU g^{-1} of talc.

For the trial, seeds of the susceptible BRS Estilo cultivar (Sementes Orient®, Patos de Minas, MG, Brazil) were used. Five bean seeds were sown per pot, and after seven days, thinning was done, leaving two seedlings per pot. The trials were conducted in a randomized block design with six repetitions. The following treatments were evaluated: formulated WP with *T. asperelloides*, formulated WP with *Beauveria bassiana*, formulated WP with *T. asperelloides* + *Beauveria bassiana*, formulated EC with *T. asperelloides*, formulated EC with *B. bassiana*, formulated EC with *T. asperelloides* + *Beauveria bassiana*. The formulations were applied in the sowing furrow, applying two mL per sowing furrow, and in the seed treatment (ST). ST was done by applying two mL to 7.5 g of seeds, and after application, the seeds remained in contact with the suspension for one hour. Controls consisted of soil infested with FOP and water application in ST and sowing furrow, and control with soil without FOP infestation and water application in ST and sowing furrow.

Plants were individually assessed for severity using the scale adopted by the International Center for Tropical Agriculture - CIAT, Cali, Colombia, as described by Schoonhoven & Pastorcorrales (1987). Severity was rated from one to nine, where 1 = plants without noticeable symptoms; 3 = plants with some wilted leaves, representing no more than 10% of the foliage, with small lesions on the hypocotyl; 5 = plants with approximately 25% of leaves showing wilt and chlorosis symptoms; 7 = about 50% of leaves exhibiting wilt, chlorosis, and limited necrosis; 9 = plants with symptoms of early necrosis, chlorosis, and wilt in 75% or more of the leaves, severely stunted plants, and dead plants.

Severity was also assessed using the scale proposed by Nascimento et al. (1995), with five severity grades: 0 = absence of symptoms; 1 = absence of external wilting symptoms and presence of vascular darkening confined to the main root; 2 = initial symptoms of chlorosis and wilting, with vascular darkening reaching the lower third of the stem; 3 = well-defined disease symptoms (chlorosis, wilting, leaf lesions, and leaf dryness) and visible vascular darkening in the middle third of the plant; 4 = well-defined disease symptoms and visible darkening in the upper third of the plant or dead plants.

Additionally, plant height was determined weekly using a graduated ruler, from the substrate surface in the pot to the apical bud of the plant, and results were expressed in centimeters. Stem diameter was measured using a digital caliper and expressed in millimeters (mm). For the evaluation of fresh and dry plant mass, the aboveground part was separated from the root system at the base of the bean plants, using scissors. The plant roots were carefully removed from the pots, then washed with a water jet to remove substrate particles without damaging the rootlets. The root system and aboveground part of the bean plants were placed in separate paper bags. For fresh mass, the bags containing the samples were weighed on a precision balance before being taken to the drying oven. For dry mass, the bags were placed in a circulating oven at a temperature of 50°C until a constant weight was obtained. After drying, the dry mass of the root system and aboveground part was determined by weighing the samples

2.12. Whitefly Control Bioassay

Bioassays were conducted to assess the pathogenicity of *B. bassiana* and *T. asperelloides* conidia on 2nd instar nymphs of the whitefly (*B. tabaci*) through normal cuticular infection via spray application, following the protocol adapted from Mascarin et al. (2013). In summary, common bean plants (*Phaseolus vulgaris* L.) were cultivated in plastic pots (350 mL) using a

mixture of soil and organic substrate enriched with mineral nutrients (Tecnutri®, Itatiba, SP, Brazil). The bean plants were grown in a screened enclosure (9 x 8 m). After 15 to 20 days of seeding, the leaves were infested with whitefly adults for approximately 24 hours, ensuring a minimum of 120 eggs laid on the underside of each leaf. Following this, adults were removed from the leaves, and infested plants were transferred to a new whitefly-free enclosure, where they remained for an additional 15 days until nymphs reached the 2nd instar (0.30 – 0.44 mm in length and 0.18 – 0.36 mm in width).

Primary bean leaves containing these nymphs were cut from the plants and transferred to ventilated Petri dishes (100 × 20 mm) covered with 15 mL of water-agar (2% w/v). Three leaves (replicates) of nymphs per fungal treatment were independently sprayed with 1 mL of fungal suspension, previously prepared with a 0.05% Tween 80 solution and calibrated to 1×10^7 viable conidia mL^{-1} using a benchtop microspray tower. The viability of these conidia was verified through colony-forming units after cultivation on PDA amended with 0.04% (v/v) Triton X-100® (Synth®) for 3 days at 28 ± 2 °C. The untreated control involved nymphs sprayed solely with a 0.09% NaCl saline solution and bean leaves without application (natural death). All treatments were maintained in a controlled environmental chamber at 28 ± 2 °C, 80 ± 11% relative humidity (RH) with a light/dark cycle of 12:12 hours. Live and mycotized (dead) nymphs were counted three and eight days post-application under a stereomicroscope at 40× magnification for survival/mortality records.

3.RESULTS

3.1. Mycelial Growth Speed Index (MGSI) and Mycelial Growth Speed Potential (%MGSI)

The isolate of *T. asperelloides* CMAA 1584 (96) exhibited the highest radial colony growth, with a diameter of 69.5 mm after three days of cultivation, attributing a MGSI of 100%. Entomopathogenic isolates showed lower MGSI compared to *T. asperelloides* CMAA 1584, with the *B. bassiana* isolate (M1) displaying an MGSI of 32.3%, representing the highest MGSI within the entomopathogenic group (Table 2).

Table 2. Mycelial Growth Speed Index (MGSI) and Mycelial Growth Speed Potential (%MGSI) of biocontrol fungi

Isolate	Species	MGSI (mm 24 h^{-1})*	%MGSI
96	*Trichoderma asperelloides*	69.5	100.0
M1	*Beauveria bassiana*	22.5	32,3
M5	*Purpureocillium* lilacinum	16.0	23.1
M7	*Beauveria caledonica*	8.8	12.7
M10	*Beauveria bassiana*	14.9	21.4
1127	*Metarhizium anisopliae*	10.1	14.5
1228	*Cordyceps javanica*	13.2	19.0
1229	*Beauveria bassiana*	13.2	19.0
CR	*Clonostachys rosea*	14.9	21.4
HT	*Hirsutella thompsonii*	7.6	10.9
47	*Metarhizium anisopliae*	9.6	13.9
351	*Beauveria bassiana*	13.9	20.0
632	*Metarhizium robertsii*	14.2	20.5

*MGSI = ((D24h – 0) + (D48h – D24h) + (D72h – D48h)) / 3. The individual percentages of MGSI (%MGSI) for the evaluated isolates were determined, considering the highest value of the mean mycelial growth speed as 100%.

3.2. Detection of chitin-degrading microorganisms

Trichoderma asperelloides CMAA 1584 (96) differed statistically from the other isolates, with a halo of 86.0 mm in both conducted assays (Table 3). Among other biocontrol fungi, *P. lilacinum* (M5) exhibited chitin degradation halos of 45.5 mm and 50.2 mm in assays 1 and 2, respectively. Isolates of *B. bassiana* (M10), *M. anisopliae* (1127 and 47), *C. javanica (*1228), *H. thompsonii* (HT), and *M. robertsii* (632) did not hydrolyze chitin.

Table 3. Detection of chitin-degrading microorganisms in solid medium containing colloidal chitin.

Isolate	Species	Diameter of the halo (mm)	
		Assay 1	**Assay 2**
96	*Trichoderma asperelloides*	86.0a	86.00a
M1	*Beauveria bassiana*	35.2c	39.2c
M5	*Purpureocillium lilacinum*	45.5b	50.2b
M7	*Beauveria caledonica*	26.0d	27.3d
M10	*Beauveria bassiana*	0.0e	0.0e
1127	*Metarhizium anisopliae*	0.0e	0.0e
1228	*Cordyceps javanica*	0.0e	0.0e
1229	*Beauveria bassiana*	31.0d	34.1c
CR	*Clonostachys rosea*	38.3c	35.5c
HT	*Hirsutella thompsonii*	0.0e	0.0e
47	*Metarhizium anisopliae*	0.0e	0.0e
351	*Beauveria bassiana*	25.0d	27.3d
632	*Metarhizium robertsii*	0.0e	0.0e

3.3. Phosphate solubilization

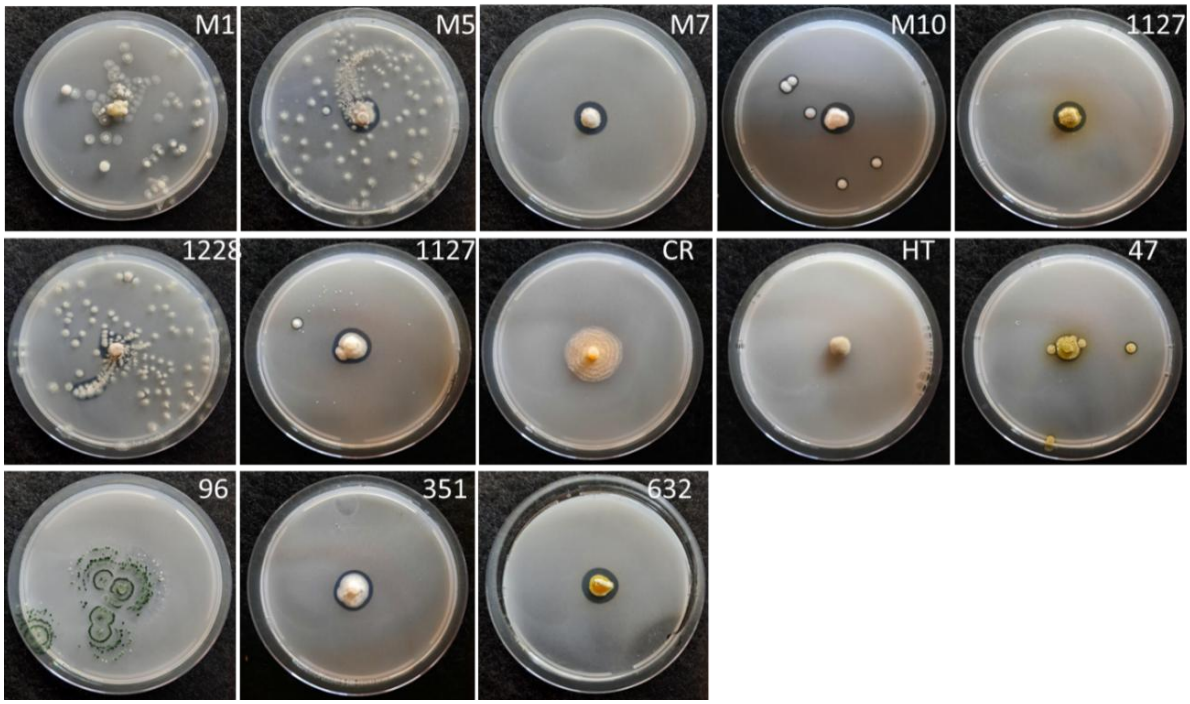
Out of the 13 isolates studied, ten solubilized phosphate in solid NBRIP medium (Table 4). Figure 1 shows the formation of a zone around the fungal colonies, indicating that the phosphate present in the culture medium was utilized by the microorganisms during the growth process.

Table 4. Phosphate solubilization by entomopathogenic isolates and *Trichoderma* in NBRIP medium (National Botanical Research Institute's Phosphate).

Species	**Isolate**	**Replications**				
		1	2	3	4	5
Trichoderma asperelloides	96	-	-	-	-	-
Beauveria bassiana	M1	-	-	-	-	-
Purpureocillium lilacinum	M5	+	+	+	+	+
Beauveria caledonica	M7	+	+	+	+	+
Beauveria bassiana	M10	+	+	+	+	+
Metarhizium anisopliae	1127	+	+	+	+	+
Cordyceps javanica	1228	+	+	+	+	+
Beauveria bassiana	1229	+	+	+	+	+
Clonostachys rosea	CR	-	-	-	-	-
Hirsutella thompsonii	511	-	-	-	-	-
Metarhizium anisopliae	47	+	+	+	+	+
Beauveria bassiana	351	+	+	+	+	+
Metarhizium robertsii	632	+	+	+	+	+

(+) = Formation of the hydrolysis halo, indicating phosphate solubilization capability. (-) = No hydrolysis halo.

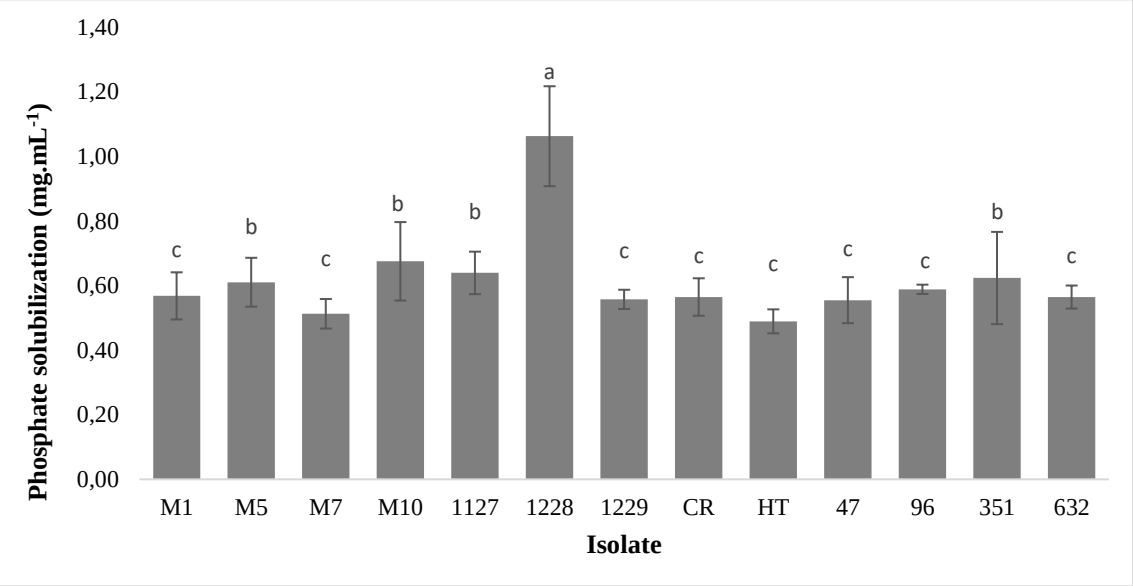
Figure 1. Phosphate solubilization by entomopathogenic isolates and *Trichoderma* in solid NBRIP medium (National Botanical Research Institute's Phosphate).



M1 = *Beauveria bassiana*. M5 = *Purpureocillium lilacinum*. M7 = *Beauveria caledonica*. M10 = *B. bassiana*. 1127 = *Metarhizium anisopliae*. 1228 = *Cordyceps javanica*. 1229 = *B. bassiana*). CR = *Clonostachys rosea*. HT = *Hirsutella Thompsonii*. 47 = *M. anisopliae*. 96 = *Trichoderma asperelloides.* 351 = *B. bassiana*. 632 = *Metarhizium robertsii*. C= controle.

Phosphate solubilization was also assessed in liquid NBRIP medium (Figure 2). The isolate of *C. javanica* (1228) solubilized 1.06 mg mL-1 of calcium phosphate ($CaHPO_4$) insoluble precipitate five days after inoculation. Isolates of *P. lilacinum* (M5), *B. bassiana* (M10 and 351), *M. anisopliae* (1127) exhibited solubilization of the insoluble calcium phosphate precipitate ranging from 0.61 to 0.68 mg mL^{-1}, with no statistically significant differences among them. The remaining isolates showed solubilization of the calcium phosphate precipitate ranging from 0.49 mg mL^{-1} to 0.59 mg mL^{-1}.

Figure 2. Phosphate solubilization in liquid NBRIP medium with the addition of insoluble calcium phosphate precipitate ($CaHPO_4$). Determination by the Murphy and Riley colorimetric method.

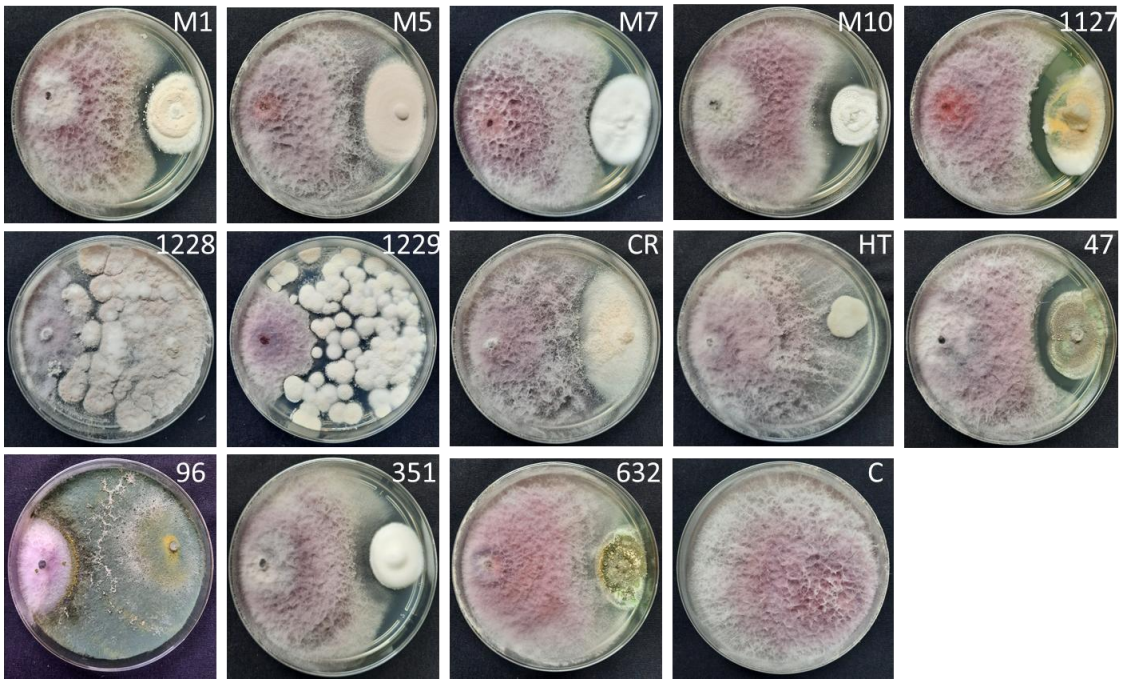


Means followed by the same letter in the columns do not differ statistically according to the Scott-Knott test at a 5% probability level. *M1 = *Beauveria bassiana*. M5 = *Purpureocillium lilacinum*. M7 = *Beauveria caledonica*. M10 = *B. bassiana*. 1127 = *Metarhizium anisopliae*. 1228 = *Cordyceps javanica*. 1229 = *B. bassiana*). CR = *Clonostachys rosea*. HT = *Hirsutella Thompsonii*. 47 = *M. anisopliae*. 96 = *Trichoderma asperelloides.* 351 = *B. bassiana*. 632 = *Metarhizium robertsii*.

3.4. Evaluation of in vitro antagonism against *Fusarium oxysporum* f. sp. *phaseoli* (FOP) Co-cultivation

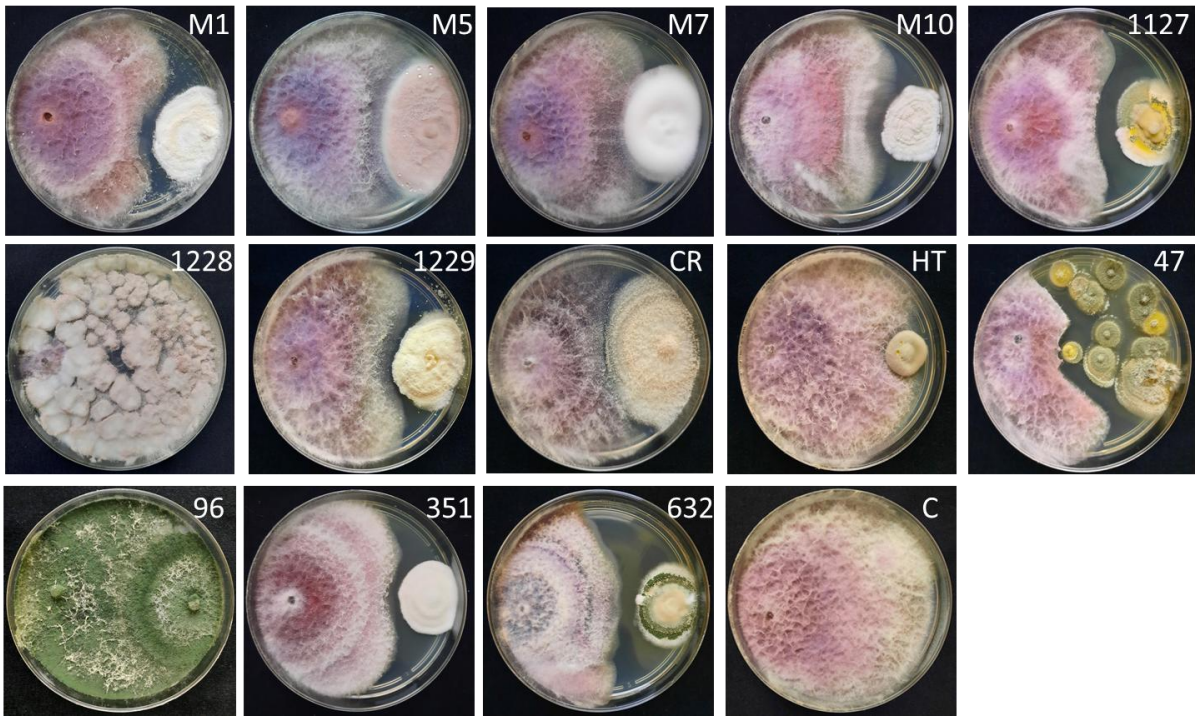
The antagonism against FOP, as assessed by the co-cultivation method, revealed that *Trichoderma asperelloides* CMA 1584 and *C. javanica* (1228) were effective in controlling FOP when inoculated simultaneously and three days after pathogen inoculation (Figure 3 and 4, Table 5). Trichoderma asperelloides inhibited 100% of the pathogen's growth when FOP was transferred three days after the antagonist's inoculation. For this isolate, it was observed that there was no formation of an inhibition halo or long-distance control, with competition for space and mycoparasitism being the probable modes of action used. These data were consistent in both evaluated assays.

Figure 3. Inhibition of growth of *Fusarium oxysporum* f. sp. *phaseoli*, isolates inoculated simultaneously.



M1 = *Beauveria bassiana*. M5 = *Purpureocillium lilacinum*. M7 = *Beauveria caledonica*. M10 = *B. bassiana*. 1127 = *Metarhizium anisopliae*. 1228 = *Cordyceps javanica*. 1229 = *B. bassiana*). CR = *Clonostachys rosea*. HT = *Hirsutella Thompsonii*. 47 = *M. anisopliae*. 96 = *Trichoderma asperelloides.* 351 = *B. bassiana*. 632 = *Metarhizium robertsii*. C= controle

Figure 4. Inhibition of growth of *Fusarium oxysporum* f. sp. *phaseoli* (FOP). Biological control agents inoculated three days before FOP.



M1 = *Beauveria bassiana*. M5 = *Purpureocillium lilacinum*. M7 = *Beauveria caledonica*. M10 = *B. bassiana*. 1127 = *Metarhizium anisopliae*. 1228 = *Cordyceps javanica*. 1229 = *B. bassiana*). CR = *Clonostachys rosea*. HT = *Hirsutella Thompsonii*. 47 = *M. anisopliae*. 96 = *Trichoderma asperelloides.* 351 = *B. bassiana*. 632 = *Metarhizium robertsii*. C= controle

C. javanica (1228), when inoculated simultaneously with FOP, inhibited 74.4% of the pathogen, which exhibited an average diameter of 23.1 mm. The same inhibition percentage (69.7%) was observed when the pathogen was inoculated three days before the Cordyceps isolate, with a mean pathogen diameter of 23.1 mm. However, when inoculated at different times, *T. asperelloides* CMA 1584 (96) differed statistically from C. javanica (1228). *Beauveria bassiana* (M1, M10, and 351) and *M. anisopliae* (1127 and 47) were significantly different from other isolates in terms of pathogen inhibition, showing the formation of inhibition halos (Table 5). For simultaneous transfer, the isolates exhibited inhibition halos of 29.5, 31.1, 28.4, 33.0, and 28.8 mm, respectively. Inoculation of the pathogen three days later maintained the results of long-distance inhibition and the formation of the inhibition halo for the previously mentioned isolates.

Table 5. *In vitro* inhibition of *Fusarium oxysporum* f. sp. *phaseoli* (FOP) growth.

Species	Isolate	At the same time			Three days later		
		Pathogen diameter (mm)	Inhibition halo	Percentage of inhibition	Pathogen diameter (mm)	Inhibition halo	Percentage of inhibition
Trichoderma asperelloides	96	23.07a	0.00b	74..5a	0.00a	0.00b	100.00a
Beauveria bassiana	M1	50.89d	29.48a	43.45d	50.62c	30.79a	43.74c
Purpureocillium lilacinum	M5	53.11d	0.00b	40.98d	48.66c	0.00b	45.92c
Beauveria caledonica	M7	52.65d	7.58b	41.50d	50.97c	0.00b	43.36c
Beauveria bassiana	M10	51.53d	31.12a	42.73d	52.60c	19.32a	41.54c
Metarhizium anisopliae	1127	48.59c	32.99a	46.01c	46.41c	33.61a	48.43c
Cordyceps javanica	1228	23.08a	0.00b	74.35a	27.26b	10.22b	69.71b
Beauveria bassiana	1229	33.05b	0.00b	63.27b	49.03c	31.62a	45.51c
Clonostachys rosea	CR	46.58c	0.00b	48.23c	42.60c	0.00b	52.66c
Hirsutella thompsonii	511	63.53e	0.00b	29.41e	62.02c	0.00b	31.08c
Metarhizium anisopliae	47	48.27c	28.8a	46.36c	39.00c	9.92b	56.66c
Beauveria bassiana	351	50.49d	28.43a	43.9d	54.04c	27.26a	39.94c
Metarhizium robertsii	632	59.27e	0.00b	34.13e	52.33c	19.95a	41.84c
Controle	C	90.00f	0.00b	0.00f	90.00d	0.00b	0.00d
C.V. (%)		5.15	37.66	6.32	13.99	71.46	15.67

Means followed by the same letter in the columns do not differ statistically according to the Scott-Knott test at a 5% probability level.

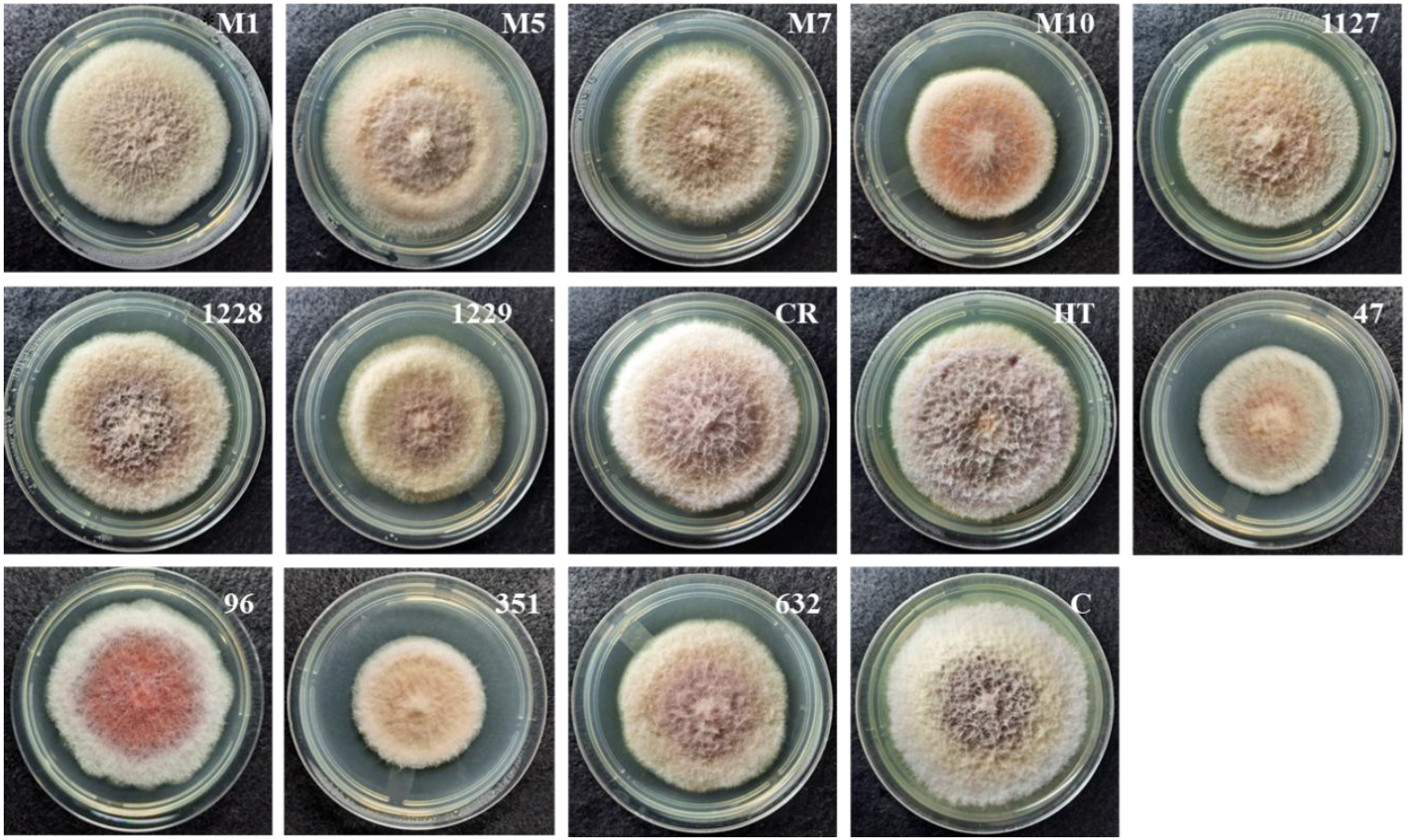
Pathogen diameter and inhibition halo in millimeters. Percentage of growth inhibition (Edington et al., 1971): Witness diameter - treatment diameter / Witness diameter * 100

3.5. Production of metabolites

The inhibition of *Fusarium oxysporum* f. sp. *phaseoli* (FOP) growth, when inoculated in culture medium enriched with 10% filtrate from the cultures of the 12 isolates studied, is presented in Figure 5 and Table 6. For the first test, all isolates significantly reduced the growth halo of FOP compared to the control. The lowest growth was observed for treatments with filtrate from *B. bassiana* (M10), *B. bassiana* (351) and *T. asperelloides* (96), with inhibition halos of 58.9, 63.0 and 63.8, respectively. The least growth inhibition for these assays occurred when the filtrates from fungi *B. bassiana* (M1), *P. lilacinum* (M5), *C. javanica* (1228), and *B. bassiana* (1229) were added to the medium (Table 6).

In the second assay, all treatments were statistically different from the control (Table 6). The treatment with filtrate from *B. bassiana* (351) and *T. asperelloides* CMA 1584 maintained the same trend as the first assay, showing a pathogen inhibition halo of 48.3 mm and 48.7 mm in diameter. For the isolate *B. bassiana* (M10), the pathogen growth halo was reduced compared to the other treatments but differed statistically from the treatment with *B. bassiana* (351) and *T. asperelloides* CMA 1584, showing a halo of 54.3 mm, equivalent to a growth reduction of 36.9%. *Purpureocillium lilacinum* (M5) showed the least reduction in pathogen growth, with a growth reduction of 17.6% in the first assay and 13.4% in the second assay.

Figure 5. Inhibition of *Fusarium oxysporum* f. sp. *phaseoli* (FOP) growth on BDA medium supplemented with 10% filtrate from biological control agents. Photos from the second assay.



M1 = *Beauveria bassiana*. M5 = *Purpureocillium lilacinum*. M7 = *Beauveria caledonica*. M10 = *B. bassiana*. 1127 = *Metarhizium anisopliae*. 1228 = *Cordyceps javanica*. 1229 = *B. bassiana*). CR = *Clonostachys rosea*. HT = *Hirsutella Thompsonii*. 47 = *M. anisopliae*. 96 = *Trichoderma asperelloides.* 351 = *B. bassiana*. 632 = *Metarhizium robertsii*. C= controle.

Table 6. Inhibition of *Fusarium oxysporum* f. sp. *phaseoli* (FOP) growth on PDA medium supplemented with 10% filtrate from biological control agents.

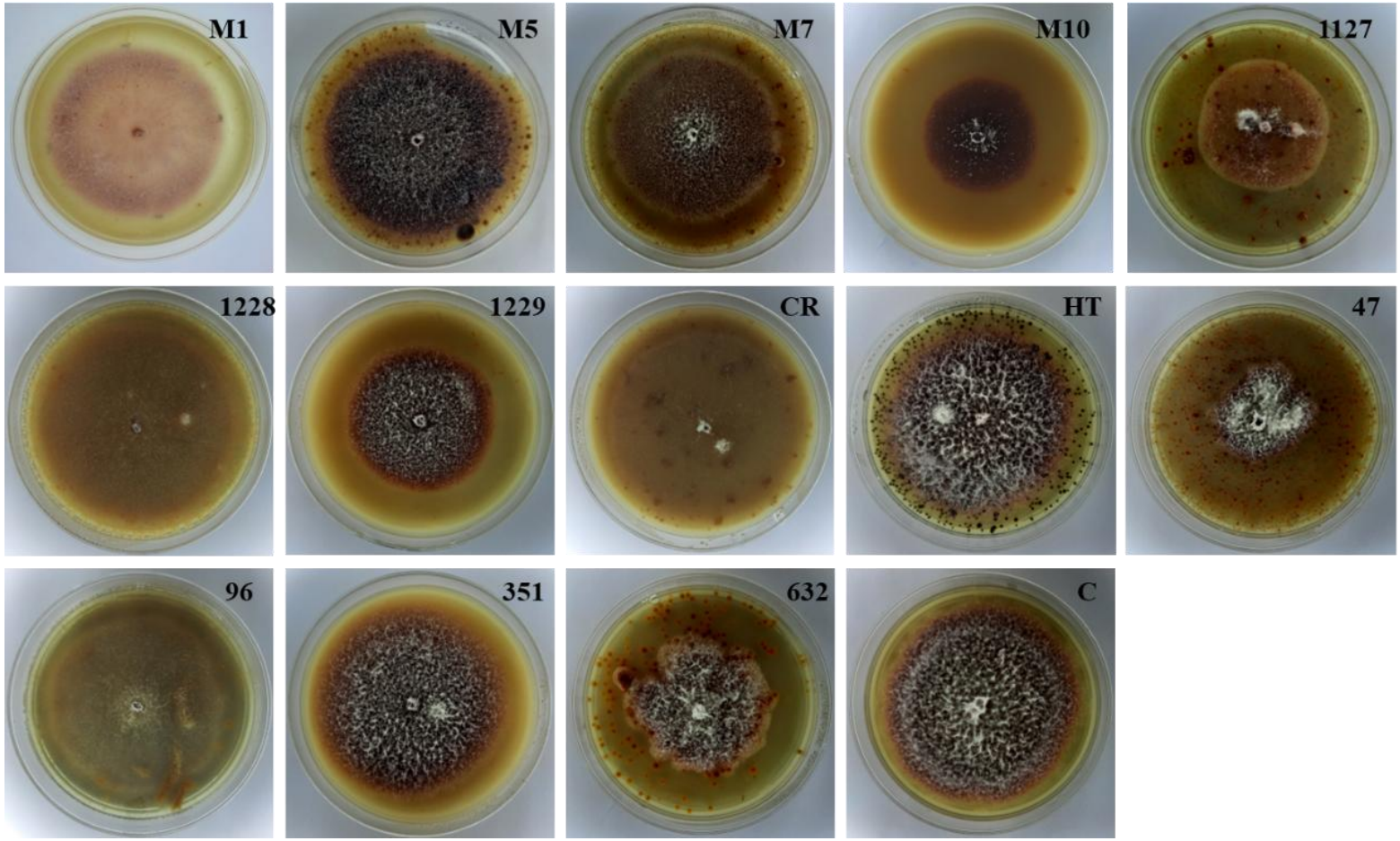
		Growth halo (mm)	
Isolate	**Species**	**Assay 1**	**Assay 2**
96	*Trichoderma asperelloides*	63.77 a	64.76 d
M1	*Beauveria bassiana*	73.15 c	64.55 d
M5	*Purpureocillium lilacinum*	70.87 c	74.46 e
M7	*Beauveria caledonica*	68.09 b	65.39 d
M10	*Beauveria bassiana*	58.91 a	54.30 b
1127	*Metarhizium anisopliae*	66.94 b	63.32 d
1228	*Cordyceps javanica*	75.28 c	63.76 d
1229	*Beauveria bassiana*	72.56 c	59.04 c
CR	*Clonostachys rosea*	69.54 b	66.20 d
HT	*Hirsutella thompsonii*	69.76 b	64.16 d
47	*Metarhizium anisopliae*	67.19 b	48.66 a
351	*Beauveria bassiana*	62.96 a	48.29 a
632	*Metarhizium robertsii*	71.76 b	63.15 d
C	Controle	86.00 d	86.00 f
CV (%)		4.67%	3.78%

Means followed by the same letter in the columns do not differ statistically according to the Scott-Knott test at a 5% probability level.

A termotolerance of the metabolites in the two conducted tests showed significant differences in the reduction of FOP colony diameter and the formation of FOP conidia (Figure 6 and Table 7). *Metarhizium anisopliae* (1127) inhibited FOP growth with a diameter of 37.2 mm, corresponding to a 56.3% reduction in growth in the first assay. The same did not occur in the second, where the percentage reduction was 20.5%, but still significantly different from the control. For conidia production, there was a reduction in both evaluated tests. *B. bassiana* (M10) reduced the pathogen's colony diameter in both assays, by 53.1% in the first and 46.67% in the second, compared to the control. The thermotolerant metabolites produced by this isolate also reduced conidia formation in both assays compared to the control (Table 7). In the second assay, the highest inhibition occurred for the *C. javanica* (1228) isolate, with a reduction of 93.1%. However, the same was not observed for the first assay.

Clonostachys rosea (CR) did not inhibit the pathogen's growth, showing a larger growth halo than the control. However, there was a reduction in conidia formation when the pathogen was grown in medium containing the culture and metabolites of this isolate. For the first assay, the concentration of recovered conidia after scraping the culture was 6.9×10^6 FOP conidia mL^{-1}, while for the control, the conidia recovery was 3.2×10^8 FOP conidia/mL.

Figure 6. Thermotolerant metabolites produced by biological control agents in the presence of *Fusarium oxysporum* f. sp. *phaseoli*. Photos from the first assay.



M1 = *Beauveria bassiana*. M5 = *Purpureocillium lilacinum*. M7 = *Beauveria caledonica*. M10 = *B. bassiana*. 1127 = *Metarhizium anisopliae*. 1228 = *Cordyceps javanica*. 1229 = *B. bassiana*). CR = *Clonostachys rosea*. HT = *Hirsutella Thompsonii*. 47 = *M. anisopliae*. 96 = *Trichoderma asperelloides.* 351 = *B. bassiana*. 632 = *Metarhizium robertsii*. C= controle.

Table 7. Thermotolerant metabolites produced by biological control agents in control *Fusarium oxysporum* f. sp. *phaseoli*

Espécie	Isolate	Assay 1			Assay 2		
		Halo (mm)	RG%**	Number of spores*	Halo (mm)	RG%	Number of spores
Trichoderma asperelloides	96	68.71c	19.36	$1.8x10^7$a	61.68e	19.59	$1.6x10^7$a
Beauveria bassiana	M1	65.69c	22.90	$7.5x10^7$b	66.30f	13.57	$8.2 x10^7$b
Purpureocillium lilacinum	M5	66.23c	22.27	$2.5x10^7$a	65.90f	14.09	$3.3x10^7$a
Beauveria caledonica	M7	62.04c	27.19	$6.1x10^7$b	60.21e	21.51	$6.19x10^7$b
Beauveria bassiana	M10	39.94a	53.12	$1.9x10^7$a	40.91c	46.67	$2.2x10^7$a
Metarhizium anisopliae	1127	37.20a	56.34	$3.0x10^7$a	61.01e	20.46	$1.6x10^7$a
Cordyceps javanica	1228	68.98c	19.40	$7.3x10^7$b	5.27a	93.12	$7.3x10^7$b
Beauveria bassiana	1229	57.51b	32.50	$3.8x10^7$a	53.80d	29.86	$1.3x10^7$a
Clonostachys rosea	CR	86.00d	-0.92	$6.9x10^6$a	86.00g	-12.11	$1.0x10^7$a
Hirsutella thompsonii	511	67.39c	20.91	$1.2x10^8$b	69.87f	8.91	$5.1x10^7$b
Metarhizium anisopliae	47	53.93b	36.70	$6.5x10^7$b	35.75b	53.39	$4.4x10^7$b
Beauveria bassiana	351	66.72c	21.69	$3.2x10^7$a	67.31f	12.25	$6.2x10^7$b
Metarhizium robertsii	632	52.13b	38.82	$1.0x10^8$b	53.63d	30.087	$5.7x10^7$b
Controle	C	85.21d	0	$3.2x10^8$c	76.71g	0	$3.2x10^8$b
C.V. (%)		5.83		47.23	4.87		41.13

Means followed by the same letter in the columns do not differ statistically according to the Scott-Knott test at a 5% probability level.

* FOP Conidia per mL. ** RG% = calculation of the percentage reduction in colony growth, calculated by the formula: RG% = (WT - TCG) / WT x 100, where WT is the diameter of the control (cm) and TCG is the total colony growth (cm).

3.6. Endophytisms and Root Colonization of Bean Plants

In tubes containing Pytagel, bean plants were developed and assessed for endophytism and root colonization. All evaluated isolates colonized the root system of the plants, except for *H. thompsonii* (HT), which did not show the formation of a turbid zone around the root. Regarding endophytisms, *B. bassiana* (M1, M10, 351), *P. lilacinum* (M5), *B. caledonica* (M7), *M. anisopliae (*1127 and 47), and *C. javanica* (1228) were endophytically isolated from both the root and the aboveground parts of treated bean plants. *Beauveria bassiana* (1229) was isolated only from the aboveground parts of the plants. *Clonostachys rosea* (CR) was isolated from the root but not from the aboveground parts. *Hirsutella thompsonii* (HT) and *M. robertsii* (632) were not recovered from any part of the bean plants (Table 8).

Table 8. Endophytisms and root colonization of bean plants developed in tubes containing Phytagel™ after seven days of sowing.

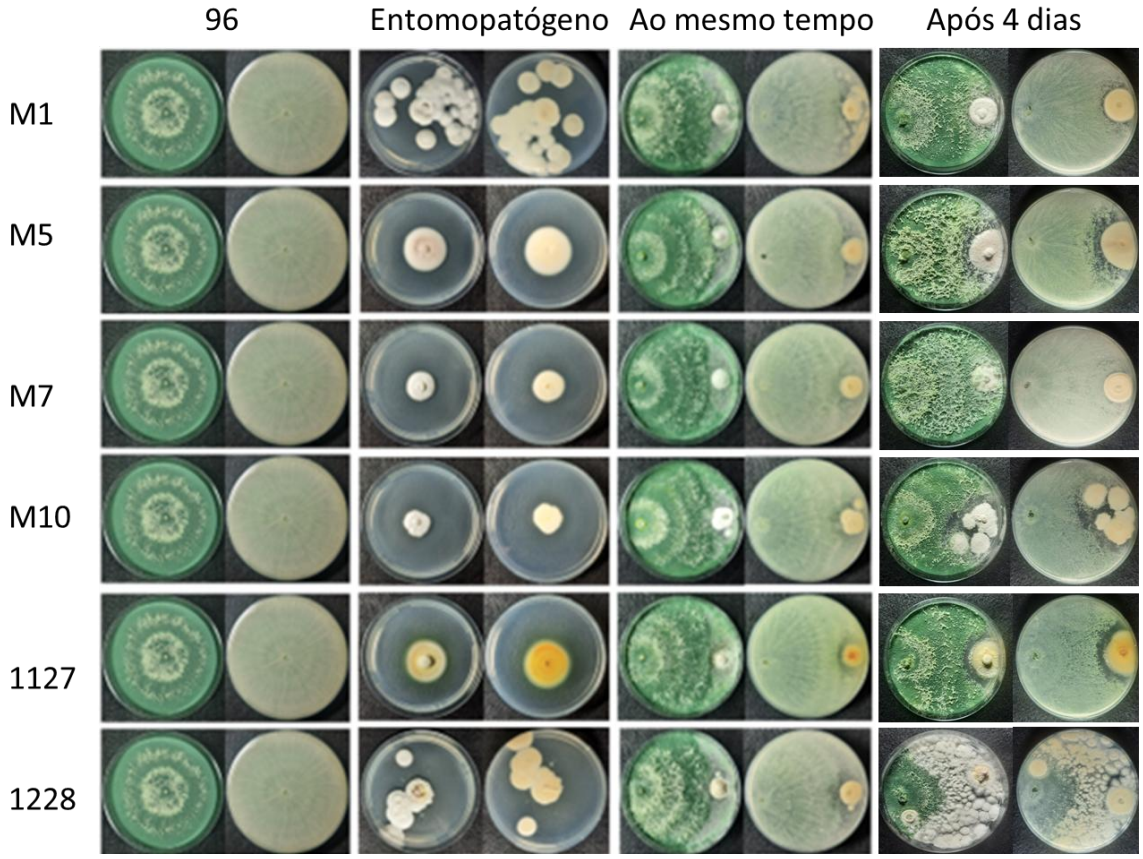
Species	**Isolate**	**Root colonization ***	**Endophytisms**	
			Root	**Aerial part**
Trichoderma asperelloides	96	+	+	+
Beauveria bassiana	M1	+	+	+
Purpureocillium lilacinum	M5	+	+	+
Beauveria caledonica	M7	+	+	+
Beauveria bassiana	M10	+	+	+
Metarhizium anisopliae	1127	+	+	+
Cordyceps javanica	1228	+	+	+
Beauveria bassiana	1229	+	-	+
Clonostachys rosea	CR	+	+	-
Hirsutella thompsonii	511	-	-	-
Metarhizium anisopliae	47	+	+	+
Beauveria bassiana	351	+	+	+
Metarhizium robertsii	632	+	-	-
Controle	C	-	-	-

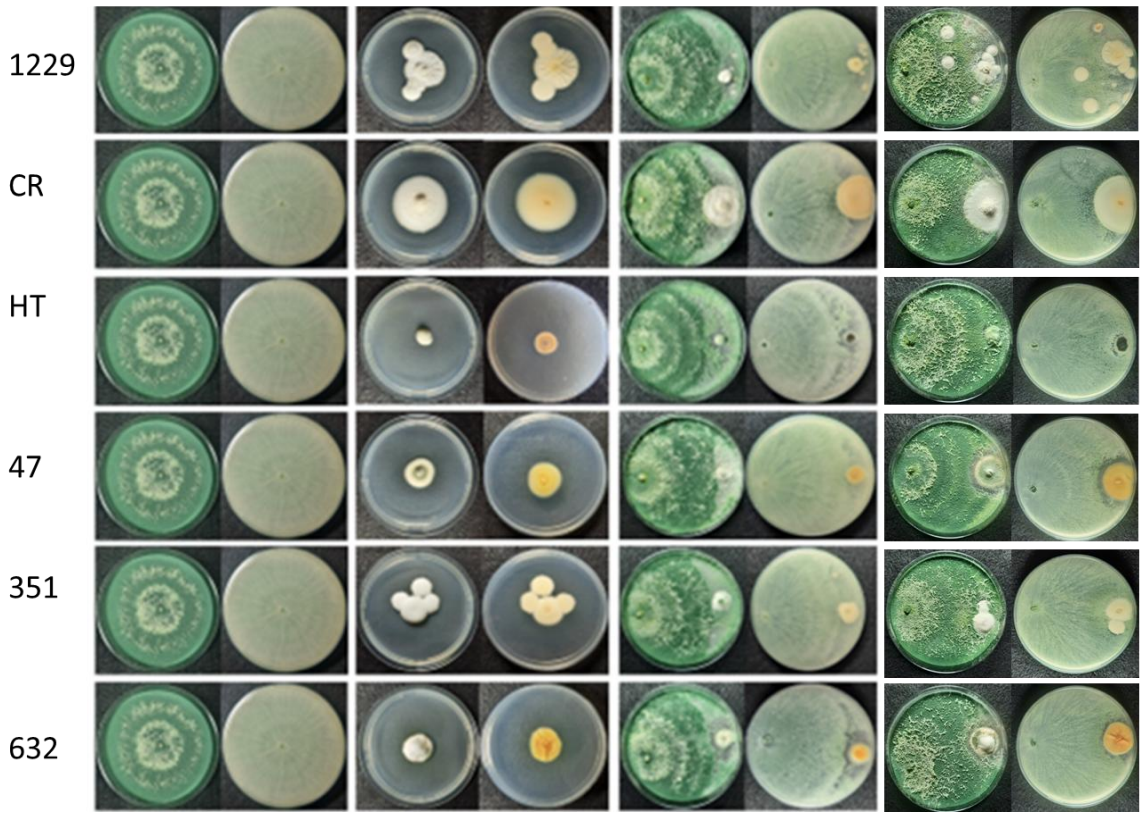
* Colonization += formation of a turbid zone around the root.

3.7. Compatibility between biocontrol agents

Compatibility between isolates of *B. bassiana* (M1, M10, 1229, and 351), M5 = P. lilacinum (M5), *B. caledonica* (M7), *M. anisopliae* (1127, 47), *C. javanica* (1228), *C. rosea* (CR), *H. thompsonii* (HT), and *M. robertsii* (632) with *T. asperelloides* CMA 1584 was verified by the co-cultivation method. The formation of inhibition halos between the confronted isolates was not observed. However, the isolate *T. asperelloides* exhibits faster growth when compared to the confronted isolates. Trichoderma asperelloides, with three days of growth, has already colonized the entire plate, preventing the growth of the other fungi studied (Figure 7).

Figure 7. Compatibility between entomopathogenic agents and *Trichoderma asperelloides* CMAA 1584 (96).



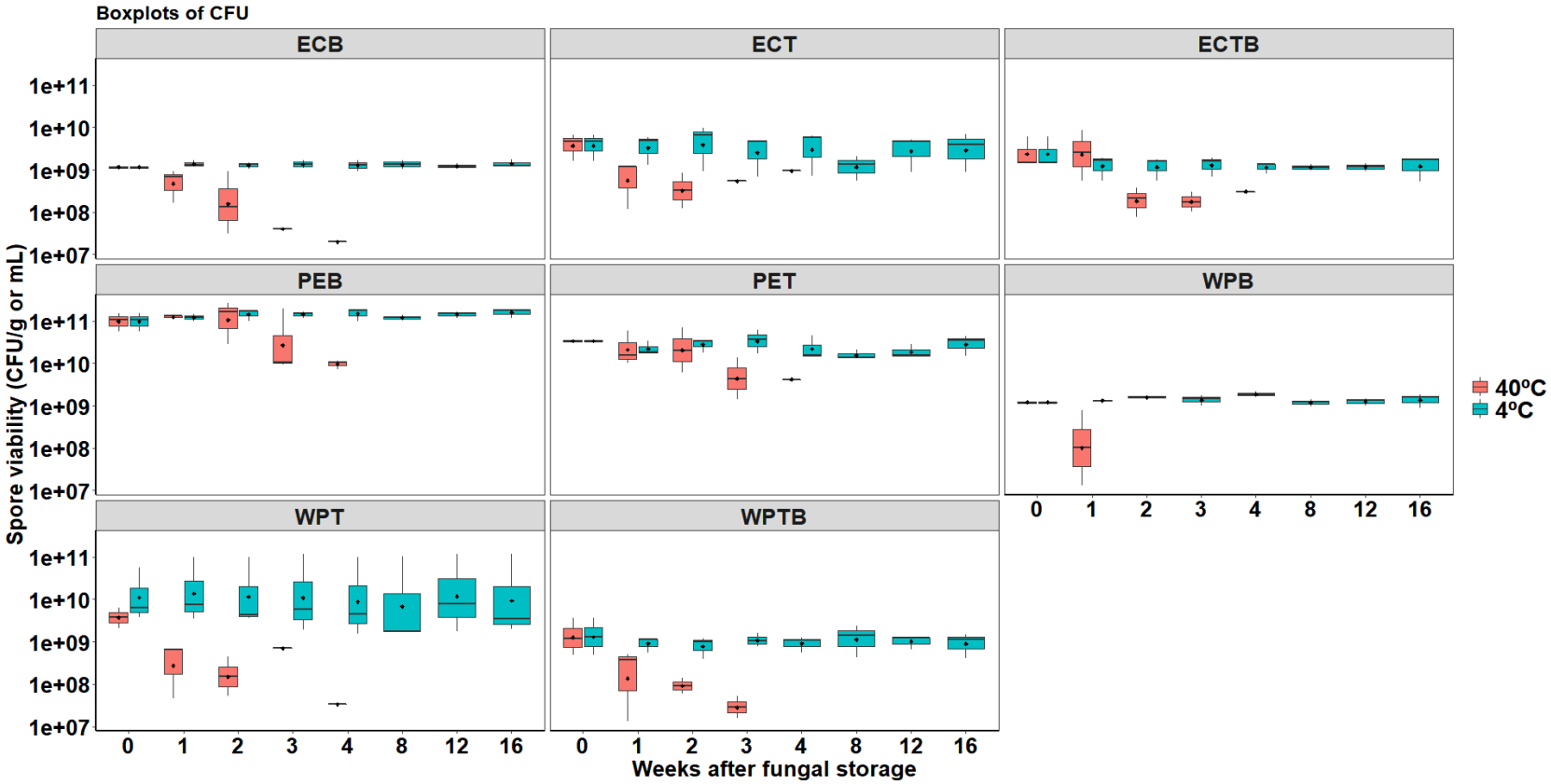


M1 = *Beauveria bassiana*. M5 = *Purpureocillium lilacinum*. M7 = *Beauveria caledonica*. M10 = *B. bassiana*. 1127 = *Metarhizium anisopliae*. 1228 = *Cordyceps javanica*. 1229 = *B. bassiana*). CR = *Clonostachys rosea*. HT = *Hirsutella Thompsonii*. 47 = *M. anisopliae*. 96 = *Trichoderma asperelloides.* 351 = *B. bassiana*. 632 = *Metarhizium robertsii*.

3.8. Storage Stability of Formulations

Both the wettable powder and concentrated emulsion formulations, for the mixture of *Trichoderma asperelloides* and *Beauveria bassiana*, as well as for the individual isolates, remained stable over the 16 weeks of evaluation when stored at a temperature of 4ºC. If there is no initial drop in the concentration of CFU g $^{-1}$. The same was not observed when the formulations were stored at 40ºC; the formulations completely lost viability in the fourth week of evaluation. Only the spore powder of *B. bassiana* stored at this temperature showed an average concentration of 9.87 x 10^9 CFU g $^{-1}$ in the fourth week, losing viability entirely by the eighth week of evaluation (Figure 8).

Figure 8. Shelf life of formulations of *Beauveria bassiana* CMAA1813 (M10) and *Trichoderma asperelloides* CMAA 1584 (96) at two storage temperatures.



PEB: Powder of *Beauveria bassiana* CMAA1813 (M10); PET: Powder of *Trichoderma asperelloides* CMAA 1584 (96); WPB: Powder of *B. bassiana;* WPT: Wettable powder of *T. asperelloides*; WPTB: Wettable powder of B. bassiana and *T. asperelloides*; ECB: Concentrated emulsion of *B. bassiana;* ECT: Concentrated emulsion of T. *asperelloides*; ECTB: Formulation in Concentrated emulsion of *B. bassiana* and *T. asperelloides*.

3.9. Control of *Fusarium* wilt in common bean

The formulations were assessed for their efficiency in controlling Fusarium wilt in common beans. In the first trial, the evaluated formulations increased root length, volume, and fresh and dry masses of the bean root system when compared to the control infested with *Fusarium* (FOP). For plant height, stem diameter, and aerial part's fresh and dry masses, there were no statistical differences among the evaluated treatments (Table 9). Regarding pathogen control, all formulations differed statistically from the control infested with *Fusarium* (FOP). The wettable powder formulation containing the mixture of Trichoderma and Beauveria showed higher control effectiveness across the three evaluated scales (Table 9).

In the second trial, the data followed the same trend, increasing root length, volume, and fresh and dry masses of the bean root system when compared to the control infested with *Fusarium* (FOP). For plant height, stem diameter, and aerial part's fresh and dry masses, there were no statistical differences among the evaluated treatments. For symptoms, all evaluat formulations were effective in controlling Fusarium wilt in common beans, differing statistically from the control infested with tathogen (FOP) (Table 10).

Table 9. Control efficiency of *Fusarium oxysporum* f. sp. *phaseoli in vivo*. Experiment conducted in February 2023.

Formulation	Height (cm)	Diameter (mm)	Root length (cm)	Root volume (mL)	FRM (g)	DRM (g)	FSM (g)	DSM (g)	Filion (2003)	Pastor-Corrales (1987)	Nascimento et al. (1995)
WPB	93.76ns	4.70ns	39.83a	13.66a	10.16a	0.86a	73.00ns	6.14ns	0.66b	3.33b	2.00b
WPT	100.19ns	4.64ns	35.50a	15.00a	12.16a	0.86a	53.20ns	6.58ns	0.05b	2.33b	1.50b
WPBT	90.91ns	4.36ns	39.25a	14.16a	12.16a	0.97a	53.20ns	7.53ns	0.00a	1.00a	0.00a
ECB	96.39ns	4.60ns	34.58a	16.66a	9.50a	0.90a	54.16ns	7.13ns	0.66b	2.66b	1.66b
ECT	92.41ns	4.48ns	37.33a	15.50a	10.50a	0.88a	51.83ns	6.47ns	1.33b	3.00b	1.66b
ECBT	107.75ns	4.77ns	42.83a	13.83a	9.66a	0.87a	55.50ns	7.04ns	0.83a	3.00b	1.50b
Controle	104.66ns	4.39ns	38.00a	15.50a	10.66a	0.87a	52.50ns	7.14ns	0.00a	1.00a	0.00a
FOP	85.87ns	4.63ns	27.25b	7.50b	5.50b	0.27b	46.33ns	5.59ns	3.33c	4.33c	2.66c
C.V.%	12.51	6.82	18.18	26.16	19.59	21.29	23.81	21.49	68.27	35.34	52.70

* Means followed by the same letter, within columns, do not differ statistically by the Scott-Knott test at a 5% probability level.** ns: not significant. FRM and DRM – fresh and dry root mass. FSM and DSM = Fresh and dry shoot mass.

Table 10. Control efficiency of *Fusarium oxysporum* f. sp. *phaseoli in vivo*. Experiment conducted in June 2023.

Formulation	Height (cm)	Diameter (mm)	Root length (cm)	Root volume (mL)	FRM	DRM	FSM	DSM	Filion (2003)	Pastor-Corrales (1987)	Nascimento et al. (1995)
WPB	103.75ns	4.63ns	42.00a	22.50a	20.66b	1.46b	51.83ns	8.71ns	0.33a	1.33a	0.33a
WPT	93.58ns	4.80ns	48.08a	22.16a	26.00a	1.72a	59.83ns	11.46ns	0.00a	1.00a	0.00a
WPBT	109.16ns	4.76ns	45.00a	22.16a	23.00a	1.79a	58.50ns	9.55ns	0.55a	1.33a	0.33a
ECB	95.10ns	4.96ns	41.00a	21.60a	21.20b	1.67a	59.40ns	9.85ns	0.00a	1.00a	0.00a
ECT	112.30ns	4.75ns	40.20a	24.20a	24.20a	1.78a	63.20ns	10.69ns	0.20a	1.40a	0.40a
ECBT	105.75ns	4.91ns	40.08a	22.66a	24.00a	1.77a	63.83ns	10.98ns	0.00a	1.00a	0.00a
Controle	108.83ns	4.66ns	41.91a	19.50a	19.00b	1.42b	57.16ns	10.46ns	0.00a	1.00a	0.00a
FOP	89.91ns	4.71ns	25.58b	12.00b	16.16b	1.01c	47.83ns	8.49ns	2.00b	2.33b	1.33b
C.V.%	16.97	6.20	18.45	23.55	20.10	14.75	20.06	29.12	183.69	14.37	193.65

*Means followed by the same letter, within columns, do not differ statistically by the Scott-Knott test at a 5% probability level. **ns: not significant. ***Data from the Pastor-Corrales scale (1987) transformed to $\sqrt{x+0.5}$. FRM and DRM – fresh and dry root mass. FSM and DSM = Fresh and dry shoot mass.

3.10. Controlling Whitefly

The studied isolates were effective in controlling whiteflies. The *Trichoderma asperelloides* isolate (96) showed higher control efficiency compared to other treatments, with a control efficiency exceeding 80% in the second count performed eight days after application. *Beauveria bassiana* (M10) exhibited a control efficiency of 65%. The application of isolates resulted in higher mortality than the control (saline solution). There was also a small rate of natural death, with approximately 20% of the flies dying naturally.

Table 11: Whitefly (*Bemisia tabaci* biotype B) control on detached bean leave

Isolate	**Mortality**		**% Mortality**	
	First count	**Second count**	**First count**	**Second count**
96	11.66a	16.33a	58.30	81.65
M10	8.66b	13.00a	43.30	65.00
Solução salina	2.33c	8.33b	11.65	41.65
Morte natural	2.16c	4.00c	10.80	20.00
C.V. (%)	22.44	22.58		

4.DISCUSSION

The results obtained reveal the capacity of entomopathogenic fungi to perform various functions, including pest control, as well as promoting plant growth and controlling plant pathogens. The entomopathogenic isolates analyzed in this study demonstrated abilities such as phosphate solubilization, endophytism, and inhibition of *Fusarium oxysporum* f. sp. *phaseoli* (FOP) growth both in vitro and in vivo. Studies by Kowalska et al. (2020) and Lacey et al. (2015) emphasize the significant importance of entomopathogenic fungi not only as biopesticides but also as biofertilizers. This functional duality makes these organisms valuable resources not only for pest control but also for fostering plant growth and comprehensively

mitigating pathogens. This multifunctional characteristic opens up horizons for new possibilities and applications in agriculture and in sectors related to crop management. The ability of entomopathogenic fungi to act as biofertilizers contributes to the environmental sustainability of agricultural systems, reducing reliance on synthetic chemicals and promoting more ecologically sustainable agricultural practices. These findings highlight the continued importance of research in this area and the potential positive impact that these biological agents can have on modern agriculture.

Phosphorus plays an essential role in plant metabolism, being a fundamental component of molecules such as ATP (adenosine triphosphate), DNA, and RNA. Previous studies, such as that of Vera-Morales et al. (2023), corroborate the importance of this element for plant development. However, its availability in the soil is often limited due to its low solubility. In this regard, phosphate solubilization is a critical process to make it accessible to plants, as highlighted by Iftikhar et al. (2024). In our study, we observed that these entomopathogenic fungi have the ability to solubilize phosphate in vitro in BRIP medium, suggesting that these microorganisms may play an important role in providing phosphorus to plants. Furthermore, studies such as that of Baron et al. (2020) support our data. In this study, strains of *Purpureocillium lilacinum* and *Metarhizium* were able to solubilize phosphate and, consequently, promote the growth of important crops such as corn, beans, and soybeans. These results suggest that the application of these fungi may represent a promising strategy to increase phosphorus availability in the soil and improve crop yields.

We also found that entomopathogenic fungi showed efficiency in controlling fusarium wilt, *in vivo*. *Fusarium oxysporum* is a pathogen that can cause diseases in various plants, notably by inducing vascular wilt (Husaini et al., 2018). Efficient control of this pathogen is vital to ensure plant health and reduce losses in agricultural production. The results obtained corroborate with previous studies in the literature, which also highlight the antagonistic potential of these agents in disease control and plant growth promotion. Research such as that of Sasan et al. (2013) demonstrated the antagonistic capacity of *Metarhizium robertsi*i against FOP, both in vitro and in vivo. Pachoute et al. (2024) report the control of *Fusarium* sp., *Alternaria burnsii*, *Epicoccum* sp., *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* in cowpea seeds (*Vigna unguiculata*) treated with entomopathogenic fungus *Beauveria bassiana*. Additionally, studies such as those of Ownley et al. (2008) and De Lira et al. (2020) emphasized the effectiveness of different species of entomopathogenic fungi in controlling various diseases and promoting plant growth,

including corn and tomato. The evidence provided by the current results, combined with literature data, highlights the versatility of these fungi and their potential for practical applications in agriculture, contributing to both soil health and the control of specific pathogens, thus promoting sustainability and effectiveness in agricultural systems.

Based on the results of the FOP control tests, *Beauveria bassiana* (M10) was chosen to be included in the consortium with *Trichoderma asperelloides* (96). In the plate test conducted to evaluate the compatibility between the isolates, no inhibition halo formation was observed between the isolates. Suryaminarsih et al. (2015) report that the compatibility between associated microorganisms may be due to the absence of secondary metabolite production by individual microorganisms to harm each other when grown in the environment. However, it was noted that the *Trichoderma* isolate exhibited a faster mycelial growth rate than the *Beauveria bassiana* isolate (Table 2). The results obtained suggest that, although there is no evidence of direct incompatibility between the isolates during in vitro tests, the difference in mycelial growth rate may indicate different competitive capacities between the two microorganisms. It is important to note that, as reported by Nunes et al. (2024), compatibility tests conducted exclusively in vitro may not be indicative of the interaction between microorganisms under field conditions. The authors also highlight that microorganisms that do not demonstrate compatibility in *in vitro* tests may, in fact, exhibit compatibility in tests conducted in vivo, where they may occupy different ecological niches (Cruz-Magalhães et al., 2022). These findings emphasize the importance of considering not only the results of in vitro tests but also broader ecological contexts when evaluating the viability and effectiveness of microbial consortia for soil-borne disease control.

The selection of the B. bassiana isolate to compose the consortium was based on the results of in vitro tests, carried out thinking both about control efficiency through the formation of an inhibition link with the pathogen, but also through the production of metabolites that are tolerant to temperature. After selection, the isolates were formulated into dispersible oil and wettable powder and evaluated for shelf life and efficacy in controlling fusarium wilt. The formulations studied showed no difference in maintaining conidial viability over the eight-month evaluation period at 4°C. In our formulations, we used sunflower oil and talc as the main vehicles, components that have low water activity as a characteristic. It is important to note that the stability of fungal formulations is influenced by several factors, including water activity and the type of vehicle used in the formulation (Barta et al., 2018; Mann and Davis, 2020; Behle

and Birthisel, 2023). Perdomo et al. (2021) observed similar results when evaluating four formulations of *Trichoderma asperellum* (wettable powder, coated granule, dispersible granule, and emulsifiable concentrate) at two different temperatures, 4°C and 30°C. In this study, maintenance of conidial viability was observed at the lower temperature, while a reduction in viability was observed at the higher temperature throughout the evaluation period. The formulations containing the consortium, as well as those containing the isolates individually, demonstrated efficacy in controlling FOP. However, we observed that there was no additive effect resulting from the mixture of microorganisms. Generally, it is expected that the consortium has superior performance compared to the application of individual strains (Nunes et al., 2024; Gehlot et al., 2021; Xu et al., 2011a). This phenomenon is often associated with synergy among the microorganisms composing the consortium, where their interaction results in a more effective combined response against the target (Stockwell et al., 2010a). However, it is important to note that formulations containing the *B. bassiana* (M10) isolate, an important pest control agent, effectively controlled FOP. In the literature, few reports of the dual control potential are available (Kang et al., 2018; Sarven et al., 2020; Sasan and Bidochka, 2013; Barelli et al., 2020). Our results are corroborated by the work of Pachoute et al. (2024). This study described an isolate of *B. bassiana* (strain PL63) that demonstrated efficacy in controlling pathogens transmitted by cowpea seeds (*Vigna unguiculata*), both in vitro and in vivo. These results underpin the effectiveness of entomopathogenic isolates in controlling plant pathogens, highlighting the promising capacity of the fungus *B. bassiana* as a dual biological control agent. The results obtained suggest the need to further investigate the dynamics and interactions within the consortium, in order to better understand how this consortium behaves in pest and disease control. As observed in Table 11, we report that the *Trichoderma* isolate demonstrated efficiency in controlling whiteflies. This report is unprecedented, as *Trichoderma* isolates are typically applied preferentially to the soil, and the assessment of their effects on pathogens and pests in the aerial part of the plant, i.e., non-target organisms, is not commonly conducted. The highlighted control efficacy underscores the need for a deeper understanding to optimize the application of these biological control agents in agricultural systems. This could pave the way for the development of more effective strategies in the integrated management of pests and diseases in agricultural environments.

5.CONCLUSION

The entomopathogenic isolates proved to be efficient in controlling FOP, phosphorus solubilization, chitin degradation, endophytic colonization, root colonization in tests carried out in vitro. The isolates of *Trichoderma asperelloides* and *Beauveria bassiana* demonstrated a dual control potential, showing efficacy in both pest control and *Fusarium oxysporum* f. sp. *phaseoli* (FOP) as well as growth promotion in bean plants, indicating their viability for integrated management of these issues. The formulations developed in this study remained stable over the evaluation period, suggesting their practical applicability. However, further research is needed to investigate the effectiveness of the consortium between *B. bassiana* and *T. asperelloides*, in order to better understand the interaction between the two isolates and the individual effects of each on the control and promotion of bean plant growth. These additional studies will help refine biological control strategies and maximize benefits for agriculture ‘.

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