MATHEUS FELIPE DE LIMA ANDREATA

BIOINSUMOS NA AGRICULTURA BRASILEIRA:
UMA AVALIAÇÃO DE BACTÉRIAS SOLUBILIZADORAS DE FOSFATO EM CONSÓRCIO COM FUNGO MICORRÍZICO ARBUSCULAR *RHIZOPHAGUS CLARUS* NA CULTURA DO ALGODÃO

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Tese apresentada ao Programa de Pós-Graduação em Microbiologia da Universidade Estadual de Londrina como parte dos requisitos para a obtenção do títulode Doutor em Microbiologia

Orientador: Prof. Dr. Galdino Andrade

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BANCA EXAMINADORA

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PRESIDENTE
Prof. Dr. Galdino Andrade - UEL

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TITULARES
Drª Ane Stéfano Simionato - Bioinput Ltda

gov.br [redacted]

Prof Dr Leandro Simões Azeredo Gonçalves - UEL

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Dr Lycio Shinji Watanabe - Bioinput Ltda

gov.br [redacted]

Dr Rafael Assis - UEPG

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RESUMO

No contexto atual de busca por uma agricultura com práticas mais sustentáveis, menor impacto ambiental e alimentos de maior qualidade nutricional, cresce a cada ano o mercado de insumos biológicos para agricultura no Brasil. Projeções indicam que esse mercado deve continuar crescendo nos próximos anos, podendo atingir 17 bilhões em 2030. Neste cenário, este trabalho teve como objetivo realizar um levantamento dos inoculantes e biodefensivos registrados no Brasil, destacando os principais microrganismos utilizados na promoção de crescimento vegetal e no biocontrole de doenças e pragas, e os seus mecanismos de ação. Com base nisso, realizamos uma discussão sobre as tendências, desafios e perspectivas futuras do mercado brasileiro de bioinsumos frente aos desafios enfrentados pela agricultura do país. Quanto aos inoculantes, com a utilização de bactérias fixadoras de nitrogênio bem estabelecida, existe uma tendência do desenvolvimento de produtos capazes de melhorar a absorção de outro nutrientes, como fósforo e potássio, sendo importante ferramenta para a redução da dependência de fertilizantes químicos, além de cepas eficientes na resistência contra estresses abióticos, que devem ser um dos grandes desafios para agricultura nos próximos anos. Em relação aos biodefensivos, um mercado que está em expansão no Brasil, o estímulo ao manejo integrado de pragas deve consolidar cada vez mais a utilização desses insumos, e a utilização de novas tecnologias, cepas multifuncionais, consórcios microbianos, otimização de bioprocessos e formulações serão fundamentais para aumentar a eficiência desses produtos e consequentemente a adesão do mercado consumidor. Além disso, com a finalidade de desenvolver um inoculante eficiente na disponibilização de fósforo no solo, prospectamos e caracterizamos *in vitro* 13 isolados bacterianos quanto a produção de ácido indolacético, produção de sideróforos, produção de exopolyssacarídeos, atividade lítica de proteínas, celulose e amido, e colonização de raízes. Dois isolados foram selecionados, identificados como *Micrococcus* sp cepa BE1 e *Lysinibacillus* sp cepa SF1, avaliadas quanto ao perfil de produção de ácidos orgânicos *in vitro* e a capacidade promover crescimento em casa de vegetação na cultura do algodão, com base em aspectos como: biomassa de raiz e parte aérea, altura, entrenó e teores de fósforo (P) e nitrogênio (N). As cepas foram inoculadas de forma isolada e em associação com o fungo micorrízicos arbuscular *Rhizophagus clarus.* A duas cepas apresentaram uma produção significativa de ácido málico e ácido glucônico em meio suplementado com fosfato tricácico. Quanto ao experimento em casa de vegetação, o tratamento contendo a cepa SF1 de *Lysinibacillus sp.* aumentou em 63% os teores de P das folhas algodão em relação ao controle, enquanto a cepa BE1 de *Micrococcus sp,* demonstrou aumento de 20% nos teores de nitrogênio. Porém, não demonstraram efeito de promoção de crescimento significativo, quando aplicadas de forma isolada e quando coinoculadas com *R. clarus*.

PALAVRAS-CHAVE: Inoculantes; biodefensivos; PGPR; PGPF.

Andreata, Matheus Felipe de Lima. Bioinputs in brazilian agriculture: an evaluation of phosphate-solubilizing bacteria in consortium with the arbuscular mycorrhizal fungus *rhizophagus clarus* in cotton cultivation. 95p. PhD Thesis in Microbiology – Postgraduate Program in Microbiology, State University of Londrina, Londrina, 2025.

ABSTRACT

In the current context of seeking agriculture with more sustainable practices, lower environmental impact, and higher nutritional quality foods, the market for biological inputs in agriculture has been growing annually in Brazil. Projections indicate that this market will continue to expand in the coming years, potentially reaching 17 billion by 2030. In this scenario, this study aimed to conduct a survey of registered inoculants and biopesticides in Brazil, highlighting the main microorganisms used in promoting plant growth and in the biocontrol of diseases and pests, as well as their mechanisms of action. Based on this, we discussed trends, challenges, and future perspectives of the Brazilian bio-inputs market in light of the challenges faced by the country's agricultural sector. Regarding inoculants, with the well-established use of nitrogen-fixing bacteria, there is a growing trend in the development of products capable of improving the absorption of other nutrients, such as phosphorus and potassium. These products are important tools for reducing dependence on chemical fertilizers. Additionally, efficient strains that enhance resistance to abiotic stresses are expected to play a crucial role, as abiotic stress will be one of the major challenges for agriculture in the coming years. As for biopesticides, a market that is expanding in Brazil, encouraging integrated pest management should increasingly consolidate the use of these inputs. The adoption of new technologies, multifunctional strains, microbial consortia, optimization of bioprocesses, and improved formulations will be essential to increasing the efficiency of these products and, consequently, driving greater market adoption. Furthermore, to develop an efficient inoculant for phosphorus availability in the soil, we prospected and characterized *in vitro* 13 bacterial isolates regarding the production of indoleacetic acid, siderophores, and exopolysaccharides, as well as their lytic activity on proteins, cellulose, and starch, and their ability to colonize roots. Two isolates were selected and identified as *Micrococcus* sp. (strain BE1) and *Lysinibacillus* sp. (strain SF1). These strains were evaluated for their in vitro organic acid production profile and their ability to promote cotton plant growth in a greenhouse experiment, considering aspects such as root and shoot biomass, plant height, internode length, and phosphorus (P) and nitrogen (N) content. The strains were inoculated both individually and in association with the arbuscular mycorrhizal fungus *Rhizophagus clarus*. Both strains showed significant production of malic acid and gluconic acid in a medium supplemented with tricalcium phosphate. In the greenhouse experiment, the treatment containing the *Lysinibacillus* sp. SF1 strain increased P content in cotton leaves by 63% compared to the control, while the *Micrococcus* sp. BE1 strain demonstrated a 20% increase in nitrogen content. However, neither strain significantly promoted plant growth when applied individually or co-inoculated with *R. clarus.*

KEYWORDS: Inoculants; Bioprotectos; PGPR; PGPF.

Lista de siglas

ACC deaminase - 1-aminocyclopropane-1-carboxylatedeaminase

AIA – Ácido indolacético

ANPII – Associação Nacional dos Produtores e Importadores de Inoculantes

BSF – Bactéria Solubilizadora de Fosfato

CEPEA – Centro de Estudos Avançados em Economia Aplicada

CNA – Confederação de Agricultura e Pecuária

CONAB – Companhia Nacional de Abastecimento

Embrapa – Empresa Brasileira de Pesquisa Agropecuária

EPS - Exopolissacarídeos

FMA – Fungo Micorrízico Arbsucular

HCN – Cianeto de hidrogênio

IPEA – Instituto de Pesquisa AplicadaMAPA – Ministério da Agricultura e Pecuária

PAT - *Product AreaTreated*

PIB – Produto Interno Bruto

PNF – Plano Nacional de Fertilizantes

Tg - Teragrama

Lista de ilustrações

Lista de tabelas

SUMÁRIO

1. INTRODUÇÃO

Com vastas extensões territoriais e um clima favorável para uma grande variedade de cultivos, o Brasil desempenha um papel crucial na agricultura global, destacando-se como um dos principais produtores e exportadores de *commodities* agrícolas do mundo. Além disso, o setor agrícola brasileiro desempenha um papel central na economia nacional, gerando empregos, impulsionando o crescimento econômico e promovendo o desenvolvimento rural em diversas regiões do país. No ano de 2023, o agronegócio foi responsável por cerca de 23,8% do Produto Interno Bruto (PIB) brasileiro cerca de 2,58 trilhões de reais, sendo 1,86 trilhão no ramo agrícola e 721 bilhões no ramo pecuário (CEPEA, 2024).

A utilização de insumos químicos foi essencial para que o Brasil se tornasse uma potência agrícola e ainda é uma importante ferramenta para manutenção de produtividade, seja na disponibilização de nutrientes a partir da adubação química ou no controle de pragas por pesticidas. Porém, é conhecido o impacto negativo desses insumos sobre a qualidade do solo, água e a saúde humana, neste panorama, têm crescido as exigências por uma agricultura mais sustentável (Baweja, Kumar e Kumar, 2020; Yadav e Devi, 2017).

Quando falamos de fertilizantes químicos, o Brasil está entre os maiores consumidores desses produtos, cerca de 8% mercado global de fertilizantes, gerando um gasto anual de 25 bilhões de dólares, o que destaca uma alta dependência externa que expõe o principal setor econômico do Brasil a flutuações no mercado internacional de fertilizantes (Brasil, 2022). Os fertilizantes fosfatados apresentam as menores taxas de eficiência de aplicação, apenas 15% a 50% do fósforo aplicado é absorvido pelas plantas. Por esse motivo, os solos brasileiros contêm concentrações elevadas de fósforo em formas insolúveis, consequência de décadas de fertilização em solos ricos em ferro (Fe) e alumínio (Al), culminando na rápida imobilização desse nutriente. Estima-se que, até 2018, o Brasil tenha acumulado 33,4 teragramas (Tg) de fósforo em seus solos. Esse reservatório acumulado de fósforo possui um valor econômico estimado de 22 bilhões de dólares (Pavinato et al., 2020).

Em 2022, o Governo Federal Brasileiro lançou o Plano Nacional de Fertilizantes (PNF), com o objetivo de reduzir a importação desses insumos de 85% para 45% até 2050. O desenvolvimento de insumos biológicos mais eficientes é uma das estratégias delineadas no plano (Brasil, 2022).

Na última década, a utilização de produtos biológicos na agricultura, com destaque para os microrganismos, tem se tornado cada vez mais comum, sendo um recurso essencial para cultivos mais sustentáveis. A expectativa é que o mercado de biodefensivos que em 2021 girava em torno de 1,3 bilhão de reais, atinja um valor de 17 bilhões em 2030, esse avanço tem sido impulsionado por políticas públicas que incentivam a pesquisa e desenvolvimento, registro de novos produtos biológicos, além de uma grande adesão do mercado consumidor (Borsari e Vieira, 2022).

A inoculação de fungos micorrízicos arbusculares (FMA) e bactérias solubilizadoras de fosfato (BSF) é uma alternativa importante para aumentar a eficiência da absorção de fósforo, reduzindo a necessidade de aplicação e utilizando o fósforo acumulado no solo (Begum et al., 2019; Bhupenchandra et al., 2024; Li et al., 2023). No entanto, a aplicação desses microrganismos ainda não está amplamente disseminada no Brasil. Por exemplo, os produtos de FMA disponíveis são importados e não utilizam cepas nativas do país, destacando uma lacuna no investimento em tecnologias para pesquisa de cepas e no desenvolvimento de sistemas de produção desses microrganismos. Quanto as bactérias solubilizadoras de fosfato, apesar de apresentarem maior disponibilidade de produtos no mercado, ainda tem baixa adesão do mercado consumidor.

É importante a busca por novas tecnologias que aumentem a eficiência desses produtos no campo. A coinoculação de FMA e BSF é uma alternativa, visto que os FMA, além de promoverem diversos benefícios diretos a planta, também estimulam a microbiota ao redor de suas hifas, a partir da maior formação de agregados e consequentemente maior retenção de nutrientes, e pela liberação de sinalizações químicas através das hifas (Mirzaei Heydari, Brook e Jones, 2024; Nacoon *et al.*, 2020; Wahid *et al.*, 2016; Wang, George e Feng, 2024). São necessárias pesquisas que busquem investigar a interação entre cepas de FMA e BSF, visando uma atuação sinérgica a fim de aumentar a eficiência da absorção do fósforo no solo pelas plantas.

2. OBJETIVOS

2.1. OBJETIVO GERAL

Analisar o atual panorama do mercado de produtos microbiológicos na agricultura brasileira e discutir sobre as tendências e perspectivas futuras desse mercado no Brasil. Em paralelo, prospectar novas cepas de bactérias solubilizadoras de fosfato e avaliar a sua coinoculação com o FMA *Rhizophagus clarus* na cultura do algodão.

2.2. OBJETIVOS ESPECÍFICOS

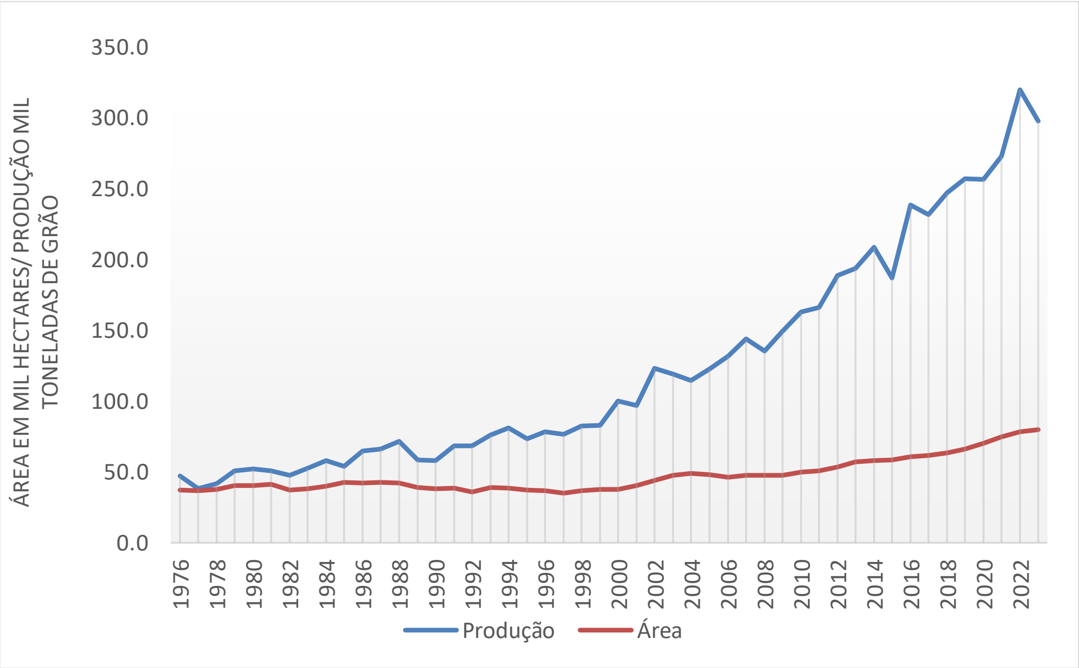
- Realizar levantamento dos bioinsumos registrados no MAPA, inoculantes e defensivos, ano de registro, formulações, alvos, mecanismos de ação e agentes biológicos;
- Discutir o histórico e o panorama atual do mercado brasileiro de bioinsumos no Brasil, desafios, tendências e perspectivas futuras;
- Prospectar isolados de bactérias capazes de solubilizar fosfato *in vitro*;
- Analisar *in vitro* características relacionadas a atividade de promoção de crescimento;
- Identificar os isolados mais promissores à promoção de crescimento vegetal;
- Avaliar os efeitos da inoculação das cepas mais promissoras, isoladas e coinoculadas com *Rhizophagus clarus* na promoção de crescimento de plantas de algodão em casa de vegetação.

3. REVISÃO BIBLIOGRÁFICA

3.1. AGRICULTURA NO BRASIL

Nas últimas cinco décadas, o Brasil se tornou um dos protagonistas da produção e exportação de produtos agrícolas. A disponibilidade de recursos naturais, aliada as políticas públicas, agricultores, ciência, tecnologia e inovação, criaram condições para que atualmente o Brasil seja um dos principais produtores de grãos do mundo, sendo a agricultura um grande impulsionador da sua economia. De 1976 a 2023, a produção de grãos cresceu de 46,9 milhões de toneladas para 297,8 milhões de toneladas, um aumento de cerca de 6 vezes. Enquanto isso a área plantada expandiu em ritmo reduzido quando contrastada com a produção, eram cerca de 36,7 milhões de hectares em 1977, comparados com 79,8 milhões de hectares em 2023 (Figura 1). Dado que evidência a intensificação da produtividade agrícola que ocorreu nesse período, o que tornou a agricultura um pilar para a manutenção do desenvolvimento econômico (Conab, 2024). Segundo o Centro de Estudos Avançados em Economia Aplicada (CEPEA), em 2023, o agronegócio foi responsável por cerca de R$ 2,58 trilhões o equivalente a 23,8% do PIB brasileiro, sendo R$ 1,86 trilhões no ramo agrícola (CEPEA, 2024).

Figura 1. Evolução da área plantada, produção e rendimento de grãos no Brasil



Fonte: Gazzoni, Cattelan e Nogueira, 2019.

Podemos associar o crescimento da produção de grãos brasileira neste período ao empenho e persistência dos produtores rurais e as políticas públicas instituídas pelo governo, como a extensão rural e crédito rural subsidiado. Além do investimento em pesquisa e desenvolvimento, que ocorrem em sua maioria em instituições públicas, como a Empresa Brasileira de Pesquisa e agropecuária (Embrapa), fundada em 1973, responsável por realizar pesquisas voltadas para o melhoramento genético, tecnologias de manejo de solo e controle de doenças e pragas. Também podemos citar a Confederação da Agricultura e Pecuária do Brasil (CNA), que teve grande relevância na disseminação de informação e capacitação de produtores rurais ao uso dessas tecnologias. Outro fator importante foi formação de agrônomos, que se intensificou nesse período com o surgimento de novas universidades, esses profissionais foram cruciais para auxiliar os produtores no processo de transição tecnológica da agricultura brasileira (Bolfe, 2018).

Ademais, a diversidade climática e geográfica do país permite a produção de uma ampla variedade de culturas de importância econômica. A soja, principal cultura, ocupa cerca de 45 milhões de hectares, dimensão que cresce a cada ano, na safra 2017/18 essa cultura cobria uma área de 35.149,2 milhões de hectares. O milho, na safra 2023/24, ocupou uma área de 20.382,2 milhões de hectares, sendo a segunda maior cultura plantada no país. Podemos citar também, outras culturas de grande importância econômica como as culturas do algodão (1.935,5 mil há), arroz (1.544,8 mil há), cana-de-açúcar (8,29 mil de há), feijão (2.860,1 mil há) e trigo (3.309,7 mil há) (CONAB, 2024). Entre essas culturas, o Brasil se destaca como líder mundial na exportação de soja (58,2% das exportações totais), café (30,8%) e açúcar (48,2%), além do suco de laranja (75,8%). Sendo o Brasil, atualmente, o terceiro maior exportador mundial de produtos agropecuários, aproximadamente USD 150,1 bilhões, atrás apenas da União Europeia e Estados Unidos (CNA, 2024).

3.2. DESAFIOS ENFRETADOS NA AGRICULTURA BRASILEIRA

3.2.1. Consumo de Fertilizantes

Atualmente, o Brasil contribui com cerca de 8% do consumo mundial de fertilizantes, quarto maior consumidor, ficando atrás apenas da China, Índia e Estados Unidos. O nutriente mais utilizado é o potássio (K), o qual o Brasil é o segundo maior consumidor, representando 38% do consumo total, seguido pelo fósforo (P), com 33%, e nitrogênio (N), com 29% (Tabela 1). Cerca de 73% desses insumos são aplicados nas culturas de soja, milho e cana-de- açúcar (Brasil, 2022).

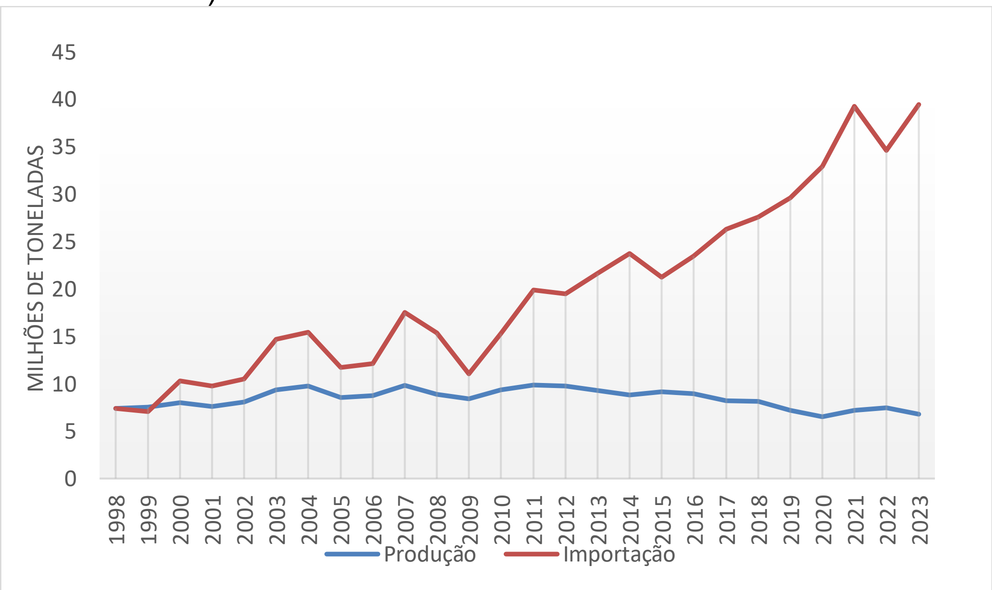
Tabela 1. Maiores consumidores mundiais de fertilizantes

	Nitrogênio (N)	**Fósforo (P)**	**Potássio (K)**
1°	China	China	China
2°	Índia	Índia	Brasil
3°	EUA	Brasil	EUA
4°	Brasil	EUA	Índia

Fonte: Brasil, 2022.

A dependência da importação de insumos é uma das grandes problemáticas na agricultura brasileira, visto que mais de 80% dos fertilizantes utilizados no país são importados. Sendo assim, a economia brasileira, tendo a agricultura como um de seus pilares, está exposta as flutuações dos valores do dólar e o mercado internacional de fertilizantes. Isso ocorre, principalmente porque a produção interna de fertilizantes não supre a demanda, apesar disso, o país tem grandes reservas das matérias-primas necessárias para produção de fertilizantes, bem como gás natural, rochas fosfáticas e potássicas e micronutrientes. Na Figura 2, podemos observar que com o passar dos anos o Brasil passou a importar cada vez mais fertilizantes químicos, visto que, houve aumento e intensificação das áreas de produção, mas a produção desses insumos não seguiu o mesmo padrão de crescimento (Brasil, 2022).

Figura 2. Produção e Importação de fertilizantes nos últimos 25 anos no Brasil (Em milhões de toneladas).



Fonte: Brasil, 2024.

Outro fator importante é a baixa eficiência do uso desses fertilizantes, tendo como exemplo o P, acredita-se que no Brasil entre o período de 1967 e 2016 as aplicações desse nutriente geraram um excedente acumulado de cerca de 33 Tg do mineral em formas insolúveis no solo. Isso ocorre principalmente porque os solos brasileiros são ácidos e ricos em alumínio e ferro, que rapidamente se ligam ao P formando complexos não disponíveis para as plantas. A adoção de práticas que visem intensificar o uso dos fertilizantes fosfatados, reduzindo a sua aplicação, podem gerar uma economia de cerca de 20,8 bilhões de dólares nas próximas décadas (Pavinato *et al.*, 2020)

Visando reduzir a dependência na importação de fertilizantes, o Plano Nacional de Fertilizantes 2050 (PNF), lançado pelo Governo Federal do Brasil no ano de 2022, apresenta medidas a serem tomadas nos próximos 28 anos com a finalidade principal de reduzir a dependência na importação de fertilizantes, de 85% para 45% até 2050. Entre as determinações do PNF para aumentar a produção de fertilizantes dentro do Brasil, estão: melhorar o conhecimento geológico do país, bem como sanar problemas de infraestrutura e espaço de armazenamento, e solucionar questões regulatórias, tributárias e ambientais. O uso de inoculantes será essencial para otimização e substituição do uso de fertilizantes, sendo de suma importância a pesquisa e o desenvolvimento de novas tecnologias voltadas para os produtos biológicos (Brasil, 2022).

3.2.2. Mudanças Climáticas

As alterações climáticas são um dos maiores desafios que o mundo enfrenta nos tempos atuais, as últimas décadas indicam que mudanças significativas no clima a nível global foram o resultado do aumento das atividades humanas que alteram a composição da atmosfera global. As projeções indicam um aumento na extensão e força dos períodos de calor extremo, juntamente com modificações na distribuição das chuvas, na disponibilidade de água e na ocorrência de secas. Essas mudanças têm o potencial de diminuir a eficiência da produção agrícola e elevar o risco de crises relacionadas à segurança alimentar (Malhi, Kaur e Kaushik, 2021).

Os impactos das mudanças climáticas já podem ser observados na agricultura brasileira, segundo a Companhia Nacional de Abastecimento (CONAB), na safra 23/24, houve uma redução de 25,7 milhões de toneladas degrãos, cerca de 8%, em relação à safra 22/23, isso se deve pela intensidade do fenômeno El Niño. Os estados das regiões do Matopiba, Centro-Oeste e parte da Sudeste do país enfrentaram falta de chuvas e altas temperaturas, enquanto na Região Sul houve excesso de chuvas, gerando atraso no plantio, principalmente da soja (Conab, 2024). Essas alterações nas condições podem, a longo prazo, causar maiores danos a produtividade das principais culturas plantadas no Brasil, soja e milho. Segundo Zilli et al., (2020) até 2050 é prevista uma redução da área cultivável de soja no país, entre 6,3% a 36,5%, enquanto para o milho a expectativa é uma redução entre 12,9% e 29,4%.

As mudanças climáticas têm afetado não só a agricultura brasileira, mas a vida dos brasileiros, e esse cenário seguirá sendo um desafio para os próximos anos A preservação de recursos naturais e adoção de práticas mais sustentáveis são essenciais para amenizar esses impactos.

3.3. Bioinsumos na agricultura brasileira

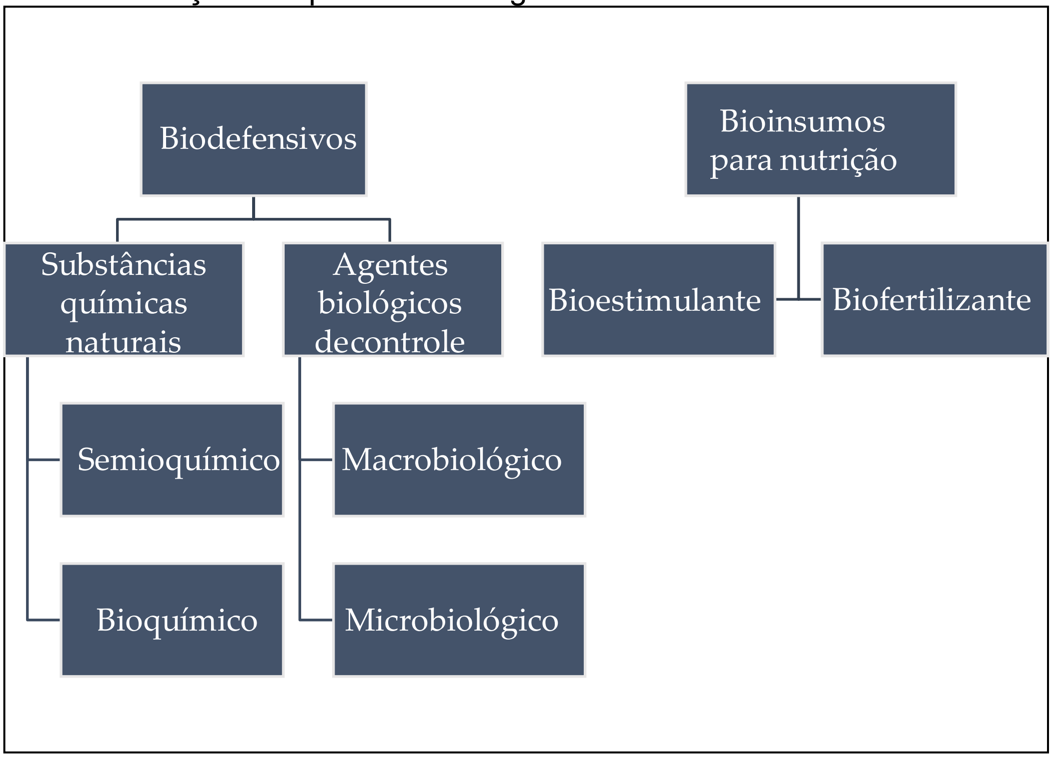
3.3.1. Classificação

Segundo o MAPA, o bioinsumo é o produto, o processo ou a tecnologia de origem vegetal, animal ou microbiana, destinado ao uso na produção, no armazenamento e no beneficiamento de produtos agropecuários, nos sistemas de produção aquáticos ou de florestas plantadas, que interfiram positivamente no crescimento, no desenvolvimento e no mecanismo de resposta de animais, de plantas, de microrganismos e de substâncias derivadas e que interajam com os produtos e os processos físico-químicos e biológicos.

Atualmente, esses produtos são divididos em duas classes, os bioinsumos voltados para nutrição vegetal e os biodefensivos, utilizados para o controle de fungos, nematoides e pragas agrícolas. Os biodefensivos, apresentam duas classes, as substâncias químicas naturais e os agentes biológicos de controle. As substâncias químicas naturais são compostos que produzem uma resposta comportamental nos organismos vivos, como feromônios e compostos aleloquímicos (semioquímico) ou agem controlando as pragas e doenças, como enzimas e hormônios reguladores do crescimento (bioquímico). Os agentes biológicos de controle são divididos em macrobiológicos (insetos, ácaros e nematoides) e os microbiológicos (vírus, bactérias, fungos e protozoários (Borsari e Vieira, 2022).

Quanto aos produtos voltados para nutrição, podem ser classificados em biofertilizantes, produtos que contém componentes ativos ou substâncias orgânicas, obtido de microrganismos ou a partir da atividade destes, bem como seus derivados de origem vegetal e animal, capaz de atuar direta ou indiretamente sobre o todo ou parte das plantas cultivadas, no aumento de sua produtividade ou na melhoria de sua qualidade, incluídos os processos e tecnologias derivados desta definição. Essas formulações são compostas por substâncias húmicas, proteínas hidrolisadas, aminoácidos, metabólitos de microrganismos e extratos de algas e vegetais. Já os inoculantes, contém microrganismos que atuam na promoção de crescimento das plantas, através de diferentes mecanismos, entre eles fixação de nitrogênio, solubilização de nutrientes, produção de hormônios de crescimento e indução de resistência a estresses bióticos e abióticos (Borsari e Vieira, 2022) (Figura 3).

Figura 3. Classificação dos produtos biológicos no Brasil

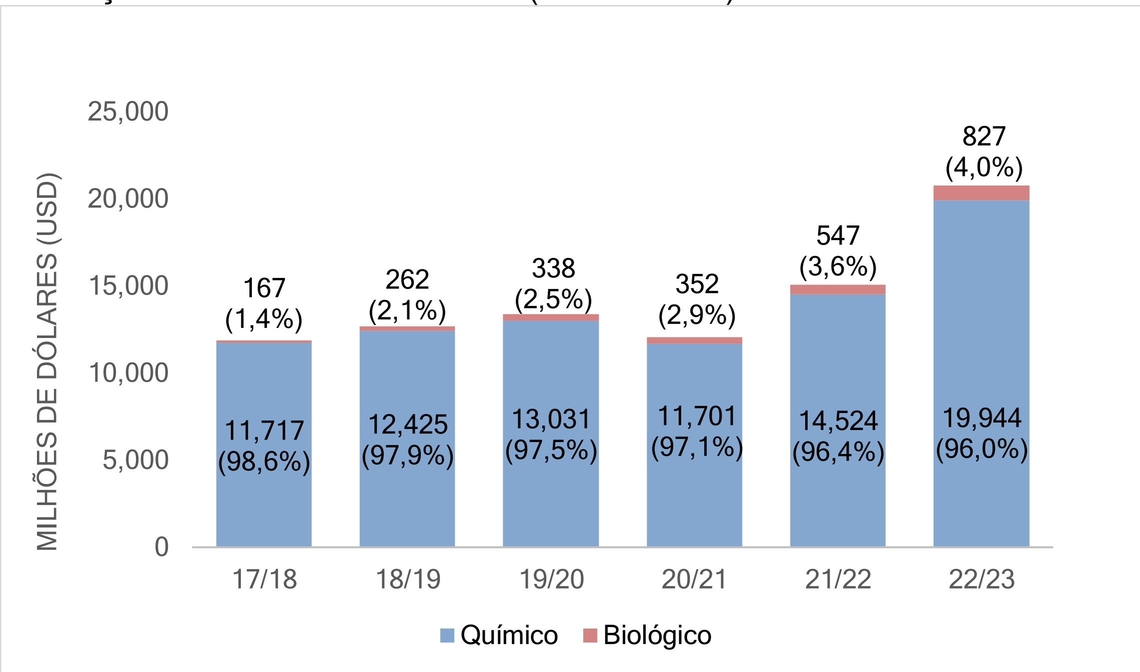


Fonte: próprio autor.

3.3.2. Mercado de Bioinsumos

O mercado de bioinsumos tem crescido a cada ano no Brasil, isso se deve principalmente pela demanda do mercado agrícola por uma agricultura mais verde, o aumento do conhecimento de empresas, cooperativas e agricultores sobre a aplicação desses produtos e a desburocratização de questões regulatórias, o que acelerou o processo de registro de inoculantes e biodefensivos. Segundo a Kynetec, na safra de 22/23 o mercado de biológicos atingiu cerca de 4% do mercado total de defesa vegetal, movimentando 827 milhões de dólares, cerca de 4 bilhões de reais, um aumento de 51% em relação ao ano interior, com uma média de crescimento de 12% nas últimas 5 safras (Figura 4). A área tratada com produtos biológicos ou *product area treated* (PAT), registrou um crescimento significativo nas principais culturas no período 2022-23. Os bioinsumos trataram uma área equivalente a 112,831 milhões de hectares no período 2022-23, frente aos 94,736 milhões de hectares na safra 2021-22. A soja correspondeu a 69% do total da PAT biológica (77,108 milhões de hectares). O milho segunda safra respondeu por 18% do total (20,6 milhões de hectares), e a cana-de-açúcar, 7%(7,5 milhões de hectares) (KYNETEC, 2024).

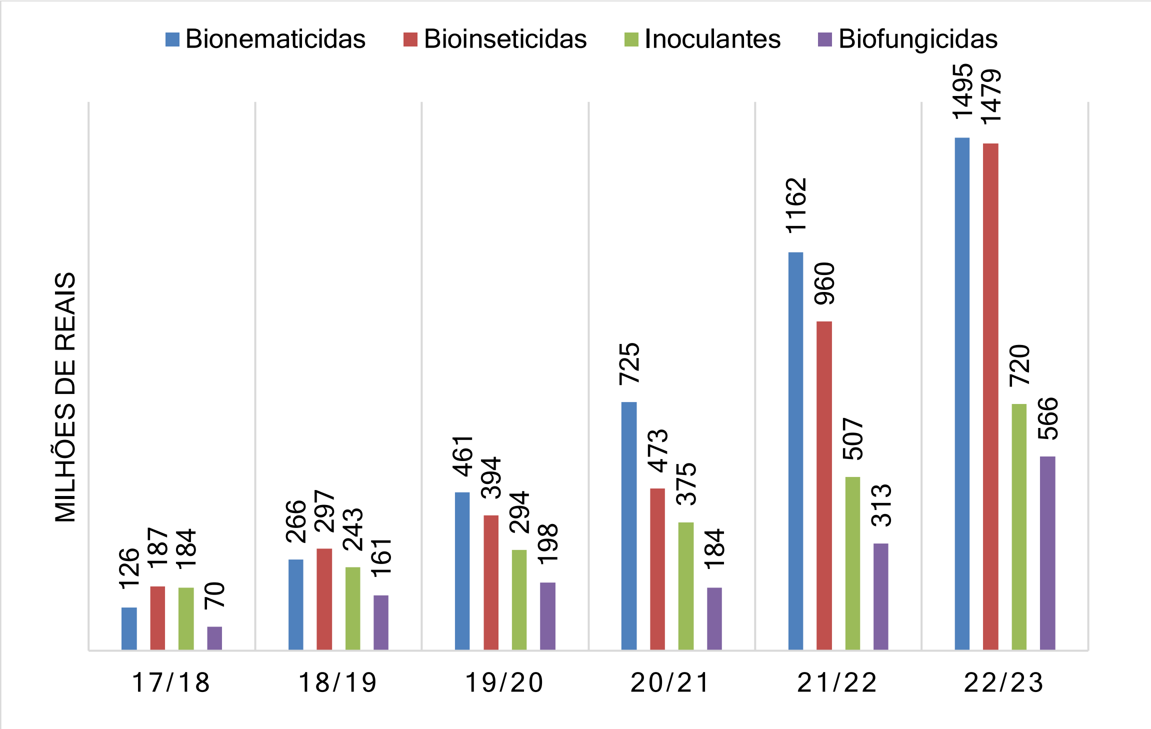
Figura 4. Evolução do mercado de biológicos vs. químicos entre as safras 17/18 até 22/23 em relação ao valor de movimentado (USD milhões).



Fonte: KYNETEC, 2024.

Os bionematicidas representam cerca de 35% do mercado de biológicos,tendo crescido de 126 milhões de reais na safra de 16/17 para 1,495 bilhão na safra de 22/23. O segundo maior mercado é o de bioinseticidas, com 35%, movimentando cerca de 1,479 bilhão de reais, enquanto os biofungicidas representam 13% do mercado de biológicos, cerca de 566 milhões na safra 22/23 (Figura 5) (KYNETEC, 2024).

Figura 5. Evolução do valor movimentado pelo mercado de biológicos entre as safras de 17/18 até 22/23 (em milhões de reais). Dividido pelos 4 principais setores: bionematicida, biofungicida, bioinseticida e inoculantes.



Fonte: KYNETEC, 2024.

A cada ano cresce também a adesão aos inoculantes, segundo a Associação Nacional de Produtores e Importadores de Inoculantes (ANPII), na safra 22/23, os inoculantes abrangeram cerca de 85% da área plantada da soja, principal cultura do país, enquanto no milho, esta área é de apenas 22% (ANPII, 2024) Um mercado que na safra 22/23 gerou cerca de 720 milhões de reais e deve continuar apresentando altas taxas de crescimento.

3.4. *Micrococcus sp*

O gênero *Micrococcus* pertence à família Micrococcaceae dentro do filo dos Actinomicetos, caracterizados como cocos Gram-positivos, não esporulantes (Britannica, 2024). São amplamente difundidas na natureza, sendo habitantes normais do corpo humano e podendo até mesmo ser essenciais para manter o equilíbrio da microbiana natural da pele. Geralmente não são patogênicas, mas algumas cepas de *M. luteus* são conhecidas como patógenos oportunistas para infecções nosocomiais capazes de causar bacteremia, pneumonia, endocardite, linfoma, artrite séptica e muitas outras doenças (Eiff, von *et al.*, 1996; Ianniello *et al.*, 2019; Khan, Aung e

Chaudhuri, 2019). Determinadas espécies são encontradas na poeira do ar (*M. roseus*), no solo (*M. denitrificans*),nas águas marinhas (*M. colpogenes*) e na pele (*M. Flavus)* (Britannica, 2024).

Os isolados normalmente apresentam pigmentação vivida, sendo reportadas colônias amarelas, laranja, verdes, rosas, vermelhas e brancas. Apesar da baixa quantidade de estudos referentes ao potencial como bioativo, existem trabalhos relatando atividade antibacteriana, bem como atividades antioxidantes, antifúngicas, anti-inflamatórias e citotóxicas (Tizabi e Hill, 2023).

Alguns trabalhos já demonstraram o potencial dessas bactérias na promoção de crescimento de plantas e biocontrole de fitopatógenos (Dastager, Deepa e Pandey, 2010; Dubey *et al.*, 2021; Raza e Faisal, 2013). A cepa de *M. luteus* AKAD 3-5m isolada a partir da rizosfera de uma variedade de soja resistente a doenças (JS-20-34), apresentou alto potencial como promotora de crescimento *in vitro*, sendo reportada a produção de auxinas e amônia, solubilização de fosfato, potencial de tolerância à dessecação (produção de biofilme e exopolissacarídeos), potencial de colonização (atividade de celulase), atividade antifúngica (quitinase, produção de cianeto de hidrogênio e atividade antagônica contra *Fusarium oxysporum* (Dubey *et al.*, 2021). Uma cepa de *M. luteus* isolada de solo florestal, foi caracterizada como produtora de auxinas, com atividade de 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase), solubilização de fosfato e produção de sideróforos. Em casa de vegetação na cultura do feijão frade, demonstrou capacidade de colonização de raiz e promoção de crescimento (Dastager, Deepa e Pandey, 2010). *M. luteus-chp37* isolada da rizosfera de plantas em solo desértico, foi capaz de promover o crescimento de plantas de milho em casa de vegetação, com ganhos de biomassa, concentração de clorofila e carotenoides (Raza e Faisal, 2013).

3.5. *Lysinibacillus* sp

Pertencente ao filo Firmicute, família Bacillaceae, as bactérias do gênero *Lysinibacillus* são Gram-positivos móveis, com células em forma de bastonete que produzem endósporos de formato elipsoidal ou esférico (Hashmi, Bindschedler e Junier, 2020). São comumente encontradas como simbiontes de plantas, bem como livres no solo. Bactérias desse gênero são conhecidas por apresentar propriedades inseticdas contra mosquitos e larvas dos gêneros *Culex*, *Anopheles*, *Mansonia* e *Aedes* (Christian et al., 2019).

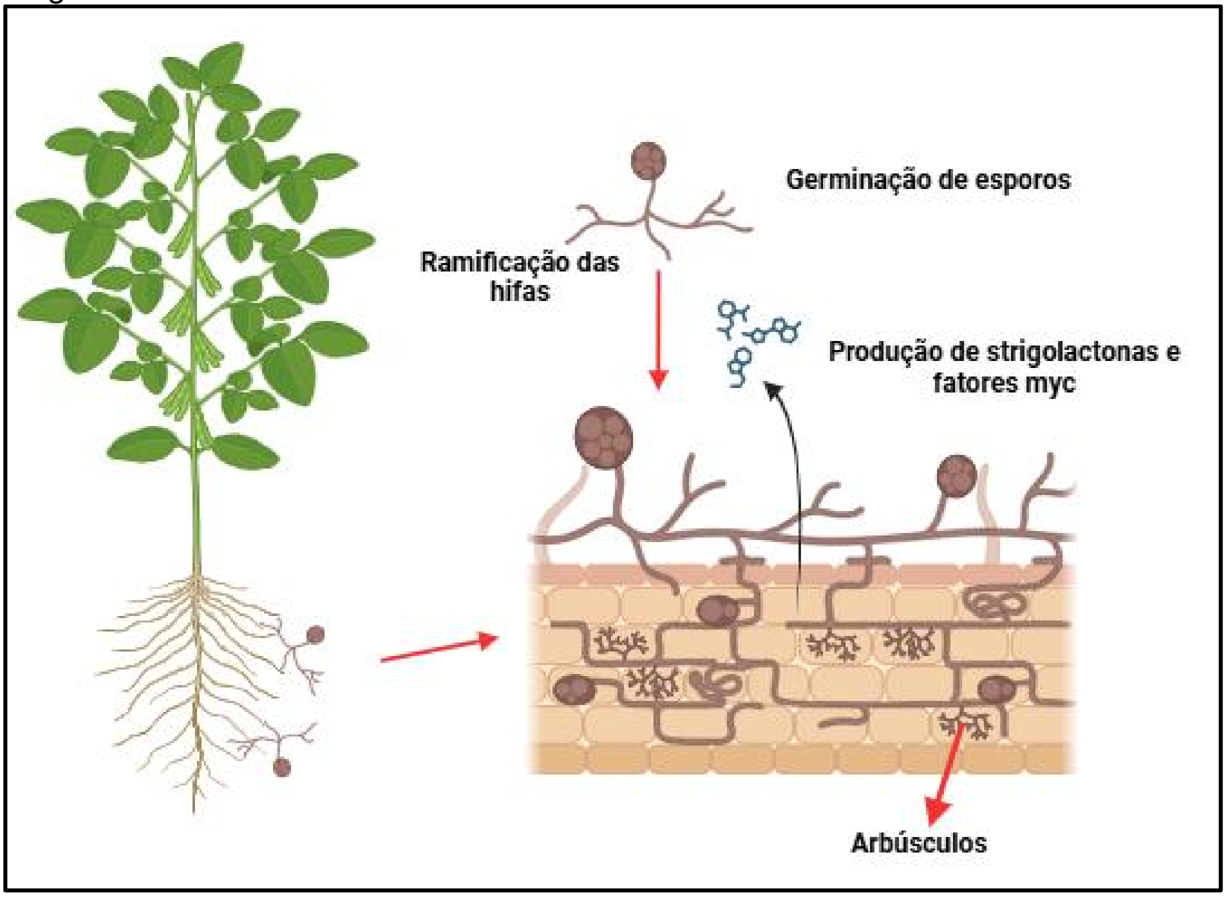
Estudos recentes têm demonstrado o potencial de algumas espécies desse gênero em promover crescimento de plantas, com destaque para *L. sphaericus* e *L. fusiformis* (Martínez e Dussán, 2018; Naureen *et al.*, 2017; Pantoja-Guerra *et al.*, 2023). A cepa Za9 de *L. sphaericus* isolada em solo rizosférico de milho, foi positiva para produção de AIA, sideróforos e cianeto de hidrogênio (HCN), também sendo capaz de solubilizar fosfato. Também foi constatada a capacidade de suprimir o crescimento de alguns fungos fitopatogênicos, devido a produção de enzimas hidrolíticas, como quitinases, lipases e proteases. Em experimento em casa de vegetação, Za9 aumentou a germinação das sementes e o crescimento das plântulas de tomate e pepino (Naureen et al., 2017). Pantoja-Guerra et al., (2023) avaliaram 20 cepas de*Lysinibacillus* produtoras de AIA, seis delas foram capazes de aumentar pelo menos uma das variáveis avaliadas em plantas de milho, em casa de vegetação. Sete cepas de *L. sphaericus* foram avaliadas como bactérias fixadoras de nitrogênio, bactérias nitrificantes e produtoras de AIA, posteriormente um consórcio utilizando as quatro melhores cepas foi capaz de aumentar o comprimento da parte aérea, o comprimento da raiz, área foliar e o número de folhas de feijão-de-porco (*Canavalia ensiformis*) em casa de vegetação e aumentar duas vezes o teor de nitrato no solo, em contraste com o controle em experimento de campo (Martínez e Dussán, 2018).

3.6. FUNGOS MICORRÍZICOS ARBUSCULARES (FMA)

Os FMA são um grupo de microrganismos do filo Glomeromycota, simbiontes obrigatórios que formam associações mutualísticas com grande parte das plantas terrestres. Esta simbiose teve origem nas primeiras plantas terrestres e acredita-se que foi essencial para adaptação das plantas ao ambiente terrestre, há pelo menos 460 milhões de anos (Declerck, Strullu e Fortin, 2005) Esses fungos formam estruturas intracelulares conhecidas como arbúsculos, sendo um conjunto de hifas altamente ramificadas que aumentam consideravelmente a superfície de contato entre o fungo e as células da planta, realizando a troca de nutrientes com a planta hospedeira (Bhupenchandra et al., 2024).

O ciclo de vida dos FMAs é caracterizado por várias fases que envolvem interações complexas entre o fungo e a planta hospedeira. O ciclo se inicia com a germinação do esporo no solo, estimulado pela presença de sinais químicos liberados pelas raízes das plantas, chamados exsudatos radiculares. Dentre eles, as estrigolactonas, fitormônios que atraem as hifas fúngicas e induzem a sua ramificação até o encontro do hospedeiro (Mitra *et al.*, 2021). Outra sinalização bem descrita, são os fatores myc, peptídeos produzidos pelos fungos, que atuam na sinalização para planta iniciar o processo de simbiose, desencadeando respostas metabólicas (López-Ráez e Pozo, 2013; Wu *et al.*, 2021). A colonização é iniciada com a formação de uma estrutura especializada chamada apressório que permite a penetração das hifas nas células da raiz. Dentro das células do córtex radicular, o fungo desenvolve os arbúsculos. Além disso, em algumas espécies de FMAs, também são formadas vesículas, que funcionam como órgãos de armazenamento. O fungo também forma uma extensa rede de hifas fora da raiz, no solo, essencial para aumentar a absorção de nutrientes, especialmente fósforo, que é difícil de ser acessado diretamente pelas raízes. O ciclo de vida se completa com a produção de novos esporos, que se formam narede hifal extrarradicular e podem persistir no solo até encontrar uma nova planta hospedeira (Smith e Read, 2008) (Figura 6).

Figura 6. Sinalização envolvida no estabelecimento da colonização intraradicial de fungos micorrízicos arbusculares.



Fonte: Próprio autor.

As atividades de FMAs sobre o crescimento vegetal é bem descrita na literatura, sendo importantes para a redução de estresses bióticos, aumentando a resistência a patógenos do solo e estresses abióticos (estresse térmico, osmótico e salino), também atuando como biorremediadores de metais pesados no solo (Begum *et al.*, 2019; Kubikova *et al.*, 2001; Mena-Violante *et al.*, 2006; Talaat e Shawky, 2014; Yooyongwech *et al.*, 2013) Esses benefícios se dão principalmente pela maior absorção de água e nutrientes através da rede de hifas extracelulares, que alcançam regiões mais profundas do solo. Também produzem fitormônios e compostos voláteis que atuam no crescimento vegetal, além da glomalina, uma substância que desempenha um papel crucial na agregação do solo.

Entre seus papéis na saúde das plantas em relação à obtenção aprimorada de nutrientes, a absorção de P é a mais completamente descrita e reconhecida como o regulador primário da associação. Em condições de altas concentrações disponíveis deste mineral no solo, a planta tende a reduzir seu investimento na associação, enquanto, sob condições de baixa disponibilidade, o investimento da planta é maior. Essa absorção aprimorada de P ocorre diretamente, por meio da absorção e transporte de P via hifas para os arbúsculos, e indiretamente, estimulando bactérias solubilizadoras de fosfato no solo (Sadhana, 2014; Smith et al., 2011; Wang et al., 2023; Zhang et al., 2021).

3.7. COINOCULAÇÃO DE FUNGOS MICORRÍZICOS ARBUSCULARES E BACTÉRIAS SOLUBILIZADORAS DE FOSFATO

A interação entre microrganismos promotores de crescimento nem sempre resulta em melhorias no desenvolvimento das plantas ou no biocontrole de patógenos; ela está sujeita a diversos fatores, como a variabilidade genética das bactérias nativas, dos hospedeiros e fatores ambientais, como luz, temperatura e matéria orgânica do solo (Meena *et al.*, 2018). Essas interações são classificadas como sinérgicas, antagônicas ou não interativas/aditivas, de acordo com o efeito observado. Sinergia ocorre quando se observa um efeito combinado, significando que a coinoculação proporciona um efeito maior em comparação com a aplicação desses agentes individualmente. Por outro lado, no caso do antagonismo, esse efeito é negativo, enquanto nas interações não interativas, não há impacto em comparação com a aplicação isolada de cada microrganismo (Anuar et al., 2023).

A coinoculação de FMA e BSF tem demonstrado resultados promissores para a melhoria da disponibilidade de fósforo no solo e o crescimento vegetal em condições de casa de vegetação e campo. Jiang et al. (2021) investigaram a interação entre FMA e BSF, mostrando que as hifas extrarradiculares dos FMAs podem transportar BSF até áreas ricas em fósforo orgânico no solo, promovendo a mineralização desse nutriente e melhorando sua disponibilidade para as plantas. Já Wahid et al. (2021) revisaram diversos estudos e relataram que a inoculação conjunta de FMA e BSF aumentou significativamente a biomassa radicular e a absorção de fósforo em culturas como milho e trigo, além de melhorar a estrutura do solo e a eficiência da absorção de água. Esses resultados reforçam a importância da combinação desses microrganismos como estratégia sustentável para otimizar a nutrição mineral das plantas e reduzir a dependência de fertilizantes químicos.

3.8. CONSIDERAÇÕES FINAIS

A agricultura brasileira está em um processo de transição para uma produção cada vez mais sustentável. Os produtos biológicos são peça-chave para esse processo e a aplicação destes tem crescido em altas taxas na última década. Pesquisas que busquem prospectar cepas mais eficientes, otimizar bioprocessos e formulações, além de viabilizar estratégias que aumentem o desempenho dos produtos biológicos em campo, são essenciais para expansão desse mercado e avanço da aplicação desses produtos no país. Dentro desse contexto, a investigação da coinoculação de microrganismos com atividade sinérgica é uma abordagem importante que pode elevar a eficiência e viabilizar a aplicação de inoculantes de microrganismos solubilizadores de fosfato.

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Artigo I

plants

Review

Microbial Fertilizers: A Study on the Current Scenario of Brazilian Inoculants and Future Perspectives

Matheus F. L. Andreata [1], Leandro Afonso [1], Erika T. G. Niekawa [1], Julio M. Salomão [1], Kawany Roque Basso [1], Maria Clara D. Silva [1], Leonardo Cruz Alves [1], Stefani F. Alarcon [1], Maria Eugenia A. Parra [1], Kathlen Giovana Grzegorczyk [1], Andreas Lazaros Chryssafidis [2] and Galdino Andrade [1,*]

[1] Microbial Ecology Laboratory, Department of Microbiology, State University of Londrina, Londrina 86057-970, Brazil; m.andreata96@gmail.com (M.F.L.A.); lafonso.bio@gmail.com (L.A.); erikatyemi@gmail.com (E.T.G.N.); julio.salomao@hotmail.com (J.M.S.); kawroquebasso.555@uel.br (K.R.B.); maria.clara.davis@uel.br (M.C.D.S.); leonardo.cruz.alves@uel.br (L.C.A.); stefanialarconf@gmail.com (S.F.A.); maria.eugenia.alcantara@uel.br (M.E.A.P.); kathlen.giovana@gmail.com (K.G.G.)

[2] Agroveterinary Sciences Center, Santa Catarina State University, Lages 88.520-000, Brazil; andreas.107@gmail.com

* Correspondence: andradeg@uel.br

Abstract: The increasing need for sustainable agricultural practices, combined with the demand for enhanced crop productivity, has led to a growing interest in utilizing microorganisms for biocontrol of diseases and pests, as well as for growth promotion. In Brazilian agriculture, the use of plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF) has become increasingly prevalent, with a corresponding rise in the number of registered microbial inoculants each year. PGPR and PGPF occupy diverse niches within the rhizosphere, playing a crucial role in soil nutrient cycling and influencing a wide range of plant physiological processes. This review examines the primary mechanisms employed by these microbial agents to promote growth, as well as the strategy of co-inoculation to enhance product efficacy. Furthermore, we provide a comprehensive analysis of the microbial inoculants currently available in Brazil, detailing the microorganisms accessible for major crops, and discuss the market's prospects for the research and development of novel products in light of current challenges faced in the coming years.

Keywords: beneficial microorganisms; co-inoculation; soil microbiome

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1. Introduction

The growing demand for sustainable agricultural practices with reduced environmental impact has led to a paradigm shift in the use of chemical fertilizers, previously considered essential for maintaining productivity. We now recognize the detrimental effects of these inputs on soil health, the environment, and human well-being. Furthermore, these fertilizers are costly and inefficient, with a significant portion of nutrients becoming immobilized in the soil, rendering them unavailable for plant uptake [1].

The use of growth-promoting microorganisms is steadily increasing, representing a more sustainable practice that can optimize soil nutrient utilization and reduce reliance on chemical fertilizers. The interaction between plants and microorganisms is fundamental for plant development. Microorganisms influence the chemical composition of the rhizospheric environment, impacting root growth, morphology, and permeability through the release of metabolites [2]. Plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF) regulate various processes, including nutrient mineralization and solubilization, the production of growth-regulating phytohormones, induction of systemic resistance, enhanced tolerance to abiotic stresses, inhibition of plant diseases through antibiotic production, reduction in ethylene in roots, production of siderophores, and iron chelation [3].

Brazil is one of the World's largest grain producers, with the agribusiness sector serving as a pillar of its economy, accounting for approximately 23.8% of Gross Domestic Product (GDP) in 2023 [4]. With the growing consumer demand for more sustainable production and products, the use of growth-promoting microorganisms has become a trend in the country, as it has globally. Additionally, the application of inoculants offers a strategy to reduce external dependence on chemical fertilizers. Due to domestic production not meeting internal demand, Brazil is a major importer of fertilizers, accounting for about 80% of the total applied, making it vulnerable to market price fluctuations [5].

According to the National Association of Inoculant Producers and Importers, 2022 saw the delivery of 134.9 million doses by associated companies, representing a notable increase of 30 million doses compared to 2021. There is a growing interest in research and development focused on novel inoculants with more effective, multifunctional strains and formulations that extend the shelf life of these products. Co-inoculation of microbial agents with diverse mechanisms of action and synergistic interactions is a prominent strategy, with a corresponding rise in product registrations in recent years. This practice was employed in 35% of the soybeans cultivated in the country during the 2022/2023 harvest [6].

In this context, this article aims to review the primary mechanisms utilized by PGPR and PGPF to promote growth (Figure 1), highlighting the co-inoculation of strains as a strategy to enhance the efficacy of biological product applications in agriculture. Based on this review, we analyzed the records of microbial inoculants in Brazil, identifying the principal microorganisms and target crops, and discussing market trends in light of current agricultural challenges.

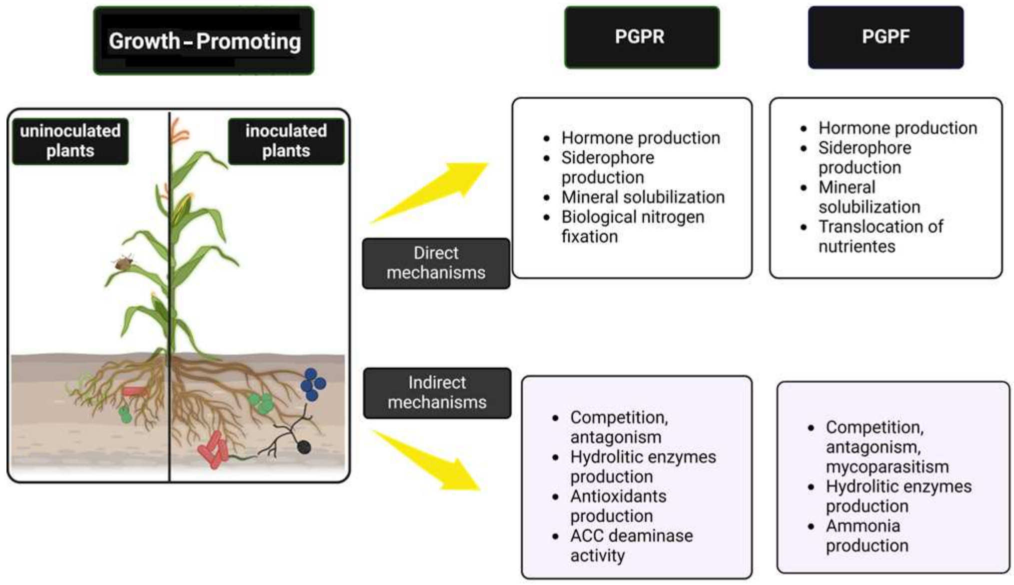

Figure 1. Direct and indirect mechanisms of PGPR and PGPF for plant growth promotion in the soil.

2. Plant Growth-Promoting Rhizobacteria (PGPR)

The beneficial interactions between plants and PGPR have emerged as a promising avenue of research for advancing sustainable agriculture and achieving more efficient cultivation practices. These bacteria can exert positive influences on plant development through a variety of mechanisms (Table 1), which will be explored in greater detail in the following sections.

Table 1. Some examples of PGPR growth promotion mechanisms.

Specie	Mechanism of Growth Promotion	Culture	Reference
Azospirillum brasilense	Nitrogen fixation	Maize (*Zea mays*)	[7]
Bradyrhizobium sp.	Nitrogen fixation	Soybean (*Glycine max*)	[8]
Pseudomonas sp.	Phosphate solubilization	Pea (*Pisum sativum*)	[9]
Pseudomonas sp.	Phosphate solubilization	Maize (*Z. mays*)	[10]
Pseudomonasbrassicae	Siderophore production	Mung bean (*Vigna radiata*)	[11]
Pseudomonas aeruginosa	Phytohormone production	Mung bean (*V. radiata*)	[12]
Pseudomonas putida	Antioxidant activity	Maize (*Z. mays*)	[13]
Bacillus velezensis	Phosphate solubilization and phytohormone production	Wheat (*Triticum aestivum*)	[14]
B. velezensis	Phosphate solubilization	Soybean (*G. max*) and Maize (*Z. mays*)	[15]
Bacillus mojavensis	Volatile organic compounds (VOCs)' production	*Arabidopsis thaliana*	[16]
Bacillus subtilis	1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity	Tomato (*Solanum lycopersicum*)	[17]
Serratia sp.	ACC deaminase activity and phytohormone production	Sunflower (*Helianthus annuus*)	[18]

2.1. Biofertilization: A Sustainable Approach to Enhance Soil Fertility

Nitrogen is a crucial limiting factor for plant growth, and its availability in the soil directly impacts agricultural productivity. Biological nitrogen fixation (BNF) has emerged as a viable strategy for reducing dependence on nitrogen-based fertilizers. BNF is a complex biological process wherein certain bacteria convert atmospheric gaseous nitrogen into nitrogenous compounds that plants can readily assimilate. This process not only supplies nitrogen to plants but also enriches the soil with essential nutrients, enhancing its fertility and structure [19]. Several genera of rhizobacteria, including *Rhizobium*, *Bradyrhizobium*, *Azospirillum*, and *Azotobacter*, are capable of fixing nitrogen and are frequently utilized as biofertilizers [20].

Rhizobium and *Bradyrhizobium* establish nodules on the roots of leguminous plants, providing the specific environment necessary for biological nitrogen fixation. These bacteria possess a unique enzyme called nitrogenase, which can cleave the triple bond of gaseous nitrogen (N_2) and convert it into ammonia (NH_3), a form readily usable by plants. The ammonia is then incorporated into essential organic molecules, such as amino acids and proteins, within the plant [21].

In contrast, *Azospirillum* and *Azotobacter* are free-living bacteria that also possess the nitrogenase enzyme, but they do not form nodules on plant roots. The conversion of N_2 molecules into NH_3 occurs within the bacterial cells themselves. The ammonia produced in this reaction can be released into the surrounding soil, thereby increasing its overall fertility [22]. BNF by rhizobacteria significantly improves soil quality, primarily by providing a continuous source of nitrogen [23].

Phosphorus (P) is another crucial element for plant growth and development. However, when phosphate-based fertilizers are applied to the soil, they are not always fully utilized by plants. Excess phosphorus often reacts with surrounding metallic cations, such as calcium (Ca^{2+}), iron (Fe^{3+}), and aluminum (Al^{3+}), forming insoluble phosphate compounds. These phosphates (calcium phosphate, iron phosphate, and aluminum phosphate) exhibit low solubility in water, restricting the availability of P to plants [24].

Bacillus, *Pseudomonas*, *Acinetobacter*, and *Pantoea* species play a crucial role in enhancing P availability in the soil, particularly in its insoluble forms. These bacteria, known as PSB (phosphate-solubilizing bacteria), produce and release various organic acids (formic, acetic, propionic, lactic, and succinic acids). The hydroxyl and carboxyl groups of these released acids react with phosphate minerals, effectively dissolving and converting them into forms that plants can readily uptake [25]. Through the production of these organic acids, these bacteria also improve the availability of other essential nutrients, such as potassium (K), a critical nutrient for plant growth. Potassium is often applied in soluble forms but tends to become rapidly fixed in the soil [26].

The combined use of nitrogen-fixing bacteria with phosphorus and potassium-solubilizing bacteria offers a viable alternative for reducing the application of chemical inputs in agricultural fertilization, contributing to a more sustainable and economically viable approach to agriculture [27].

2.2. Protection against Oxidative Stress in Adverse Environmental Conditions

Reactive oxygen species (ROS), such as the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-), are highly reactive molecules produced in plants as byproducts of oxygen metabolism, especially during photosynthesis and respiration. In stressful situations, such as drought, salinity, extreme temperatures, pathogen infection, and exposure to pollutants, ROS production increases significantly. Although at low levels they act as signaling molecules, at high concentrations they cause oxidative damage to proteins, lipids, and DNA, resulting in cellular dysfunction and, in severe cases, cell death [28].

Antioxidants play a vital role in protecting plants from oxidative stress and are classified as either enzymatic or non-enzymatic. Enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), glutathione reductase (GR), and ascorbate peroxidase (APX), neutralize ROS through specific biochemical reactions and are produced by both plants and certain PGPRs [29]. Non-enzymatic antioxidants, such as ascorbic acid (vitamin C), glutathione (GSH), carotenoids, tocopherols (vitamin E), and flavonoids, directly scavenge ROS. While these compounds are primarily synthesized by plants, some PGPRs are also capable of producing ascorbic acid and glutathione [30].

Several species of rhizobacteria, including *Bacillus pumilus*, *Bacillus subtilis*, and *Pseudomonas* sp., contribute to the protection of plants from oxidative stress. They achieve this by either directly producing antioxidants or by stimulating plants to increase their own antioxidant production [31]. This collective action of antioxidants creates a protective network that shields plant cells from oxidative damage, ensuring the maintenance of plant health and growth even in adverse environmental conditions [32].

2.3. Production of Phytohormones by Rhizobacteria

Phytohormones are naturally occurring chemical substances within plants that regulate a broad spectrum of physiological and developmental processes. Collectively, these hormones can interact synergistically or antagonistically, governing various aspects of plant growth, development, and adaptation to the environment. The principal phytohormones include auxins, cytokinins, gibberellins, abscisic acid, ethylene, salicylic acid, and jasmonates. In addition to being synthesized by plants themselves, the production of many of these hormones can be induced or directly produced by PGPRs such as *Pseudomonas*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Serratia*, and others [33].

The main growth-promoting mechanism employed by rhizobacteria is the production of auxin, particularly indole-3-acetic acid (IAA). Over 80% of bacteria associated with the rhizosphere are capable of synthesizing IAA [34]. Auxins modulate growth processes in both the aerial and root portions of plants. The increase in root size, induced by bacterial IAA, enhances the surface area available for nutrient and water uptake, thereby improving overall plant vigor [35]. Additionally, research in *Arabidopsis thaliana* has shown that auxins improve resistance to water stress by positively regulating the activity of antioxidant enzymes, aiding in the mitigation of the effects of reactive oxygen species generated under stressful conditions [36].

Cytokinins (CKs) are known to regulate a wide array of plant growth, development, and physiological traits, including seed germination, apical dominance, flower and fruit development, and leaf senescence. CKs also participate in a diverse range of responses to biotic and abiotic stresses. Although the precise mechanisms of action are not fully understood, numerous studies have observed an increased endogenous concentration of CKs in plants under stress or altered stress responses upon the addition of exogenous CKs [37]. Bacterially produced CKs can induce resistance in plants against bacterial and fungal pathogens [38]. However, they can also stimulate plant responses to herbivore attacks by promoting the expression of wound-inducible genes, leading to the accumulation of insecticidal compounds [39].

Gibberellins (GAs) are phytohormones that promote various aspects of plant growth, including stem elongation, seed germination, flowering, and fruit development. They are also crucial for breaking seed dormancy [40]. In contrast, abscisic acid (ABA) is associated with stress responses and dormancy. It inhibits growth, promotes stomatal closure to minimize water loss, and induces seed dormancy, aiding plants in surviving adverse conditions such as drought or salinity [41]. At the molecular level, GAs and ABA exert antagonistic regulation on the expression of specific genes. GAs activate genes involved in cell growth, while ABA induces genes related to stress response protein synthesis. The signaling pathways of these hormones involve specific receptors and transduction mechanisms that modulate the activity of transcription factors, leading to appropriate cellular responses. The interaction between gibberellins and ABA exemplifies the antagonistic regulation of critical processes within the plant life cycle. The balance between these hormones enables plants to adjust their growth and development in response to environmental fluctuations, ensuring survival and adaptation to changing conditions [42].

Some PGPRs play a vital role in plant health by producing and modulating plant hormones such as salicylic acid (SA), jasmonate (JA), and ethylene (ET), which are essential for plant responses to biotic and abiotic stresses. Salicylic acid (SA), produced by PGPRs like *Pseudomonas* and *Bacillus*, induces systemic acquired resistance (SAR), enhancing defenses against pathogens [43,44]. Jasmonate (JA) and ethylene (ET), influenced by PGPRs, trigger induced systemic resistance (ISR) and increase defense against herbivores. The coordinated interaction among SA, JA, and ET, mediated by PGPRs, offers significant benefits to plants. Cross-talk between these signaling pathways allows the plant to fine-tune its responses to multiple stressors, promoting both defense mechanisms and growth. While SA is most effective against biotrophic pathogens, JA and ET play crucial roles in defense against herbivores and under abiotic stress conditions. The combined effect of these responses provides a robust defense strategy against a wide range of stresses, increasing plant resilience [45,46].

The capacity of PGPRs to directly induce or produce phytohormones underscores their crucial role in promoting plant growth and enhancing stress resilience, providing promising avenues for increasing agricultural productivity.

2.4. Production of 1-Aminocyclopropane-1-Carboxylic Acid (ACC) Deaminase

Ethylene is a gaseous hormone produced by plants and plays a vital role in their development and adaptation to adverse conditions. During biotic stresses, such as pathogen attacks, the increase in ethylene levels triggers a series of defense responses, including the production of phytoalexins and the activation of resistance genes [47]. Similarly, in abiotic stresses such as drought and salinity, ethylene assists plants in survival by adjusting growth, closing stomata to reduce water loss, and promoting the synthesis of antioxidant enzymes to combat oxidative stress. However, when ethylene levels exceed what is needed, undesirable effects can occur, such as the inhibition of root and stem growth, reduction in photosynthetic rate, and even induction of premature senescence. Additionally, excess ethylene can lead to premature leaf and flower drop, compromising the plant's ability to photosynthesize and reproduce. Therefore, the precise regulation of ethylene levels is crucial to ensure the optimal growth and development of plants [48].

Some PGPR produce ACC deaminase, an enzyme that reduces ethylene concentrations in the plant by cleaving the precursor ACC into ammonia and α-ketobutyrate. This mechanism helps reduce damage caused by various stresses, particularly environmental ones, such as in saline soils [49], heavy metals [50], and water stress [51].

2.5. Chemical Signals: Volatile Organic Compounds (VOCs)

Volatile organic compounds (VOCs) are small chemical substances (<300 Da) widely released by most living organisms. Bacterial VOCs encompass a variety of categories, including alcohols, benzenoids, aldehydes, alkenes, acids, esters, terpenoids, and ketones. The ones produced by rhizobacteria can trigger several intra- and interspecies responses; in plants, we highlight the plant growth promotion and induction of systemic resistance effects [52].

Once liberated, these compounds become powerful chemical signals displaying roles in the modulation of microbial community growth, movement, and virulence; the expression of genes related to the management of stress; secondary metabolites production; and quorum sensing. Plant responses to VOCs include growth (shoot and roots, especially), enhanced nutrient uptake, and induced systemic resistance. Animal responses to microbial VOCs include the attraction of beneficial insects and repulsion of harmful pests [52,53].

Among the genera of rhizobacteria identified as VOC producers are species of *Bacillus*, *Serratia*, *Enterobacter*, and *Pseudomonas* [54]. On the suppressive effects of bacterial VOCs, for example, molecules produced by *Pseudomonas* are reported to impair *Ralstonia solanacearum* growth and virulence [55], suppressive effects were also reported over *Rhizoctonia solani*, *Botrytis cinerea*, and *Phytophthora cinnamomi*, impairing the different process of the phytopathogenic fungi [56]. Examples of beneficial effects from VOCs produced by fungi are presented in Table 2.

Table 2. Some examples of PGPF growth promotion mechanisms.

Specie	Mechanism of Growth Promotion	Culture	Reference
Trichoderma sp.	Phosphate solubilization	Soybean (*Glycine max*)	[57]
Trichoderma sp.	VOCs production	*Arabidopsis thaliana*	[58]
Trichoderma koningiopsis	VOCs production	*A. thaliana*	[59]
Trichoderma viride	VOCs production	Tomato (*Solanum lycopersicum*)	[60]
Trichoderma asperellum	Phosphate solubilization and phytohormone production	Cucumber (*Cucumber sativus*)	[61]
Rhizophagus clarus	Increase in P and N content	Soybean (*G. max*) and Cotton (*Gossypium hirsutum*)	[62]
Glomus intraradices	Salt tolerance	Wheat (*Triticum aestivum*)	[63]
Rhizophagus irregularis	Drought tolerance	Maize (*Zea mays*)	[64]
Mix of *R. clarus*, *R. intraradices*, *Septoglomus deserticola*, *Funneliformis mosseae*	Water deficit tolerance	Soybean (*G. max*)	[65]
Rhizophagus irregularis, *F. mosseae*, and *Funneliformis geosporum*	High temperature tolerance	Soybean (*G. max*)	[66]

The multiple categories of compounds and the intricate biochemical pathways involved in their signaling make the complete elucidation of these mechanisms a challenge. However, studies indicate that microbial VOCs are strongly associated with promoting plant growth, possibly by modulating the synthesis and/or metabolism of phytohormones produced by rhizobacteria or by the plants themselves [33].

2.6. Production of Siderophores

Siderophores are secreted molecules that can be used both as biofertilizers and/or biocontrollers. As biofertilizers, they optimize the availability of iron in the root zone, and at the same time, their biocontroller activity relies on competing with phytopathogens for access to iron [67].

From a structural perspective, a typical siderophore presents one or more well-known functional groups such as hydroxamate, catecholate, or carboxylate. These functional groups establish bonds with iron ions, forming stable complexes. Although siderophores are widely recognized for their affinity with iron, it is important to emphasize that some of these organic molecules can also form complexes with other metals, including zinc, copper, manganese, and others [68].

There are two theories regarding how plants absorb iron from microbial siderophores. According to the first theory, siderophores with high redox potential donate iron (Fe^{2+}) to the plant's transport system after being reduced. This happens when siderophores with Fe^{3+} are transported to the roots, where reduction occurs in the apoplast, retaining Fe^{2+} and increasing iron concentrations in the roots. Following the second theory, microbial siderophores can bind to iron in the soil and exchange ligands with plant phytosiderophores, a process dependent on various factors such as complex stability, concentrations, and root environment conditions [69].

In addition, the high affinity of siderophores for iron results in limiting the access of this element by other microorganisms, suppressing the population of phytopathogenic agents in the rhizosphere. The reduction in disease incidence strengthens plant health and reduces the need for high doses of chemical control agents [70].

3. Plant Growth-Promoting Fungi (PGPF)

Some genera of saprophytic fungi present in the rhizosphere are capable of promoting growth by colonizing the plant's roots, primarily through increased nutrient absorption, phytohormone production, and the induction of systemic resistance. Among the most common are *Aspergillus*, *Fusarium*, *Penicillium*, *Piriformospora*, *Phoma*, and *Trichoderma* [71]. Here, we focus on fungi from *Trichoderma* genus and arbuscular mycorrhizal fungi (AMF). The latter are obligatory symbionts of plant roots and have a notable effect on nutrient acquisition and resistance to abiotic stress [72] (Table 2).

3.1. Trichoderma spp. as Biocontrol Agents and Fertilizers

Fungi of the genus *Trichoderma* have remarkable abilities in the biological control of pathogenic fungal agents that harm plant growth. This activity in controlling fungi is well documented in the literature, and various mechanisms of action are described [73].

This effect can occur through direct or indirect mechanisms. Direct mechanisms rely on competition for resources, such as space and nutrients. *Trichoderma* spp. colonize surfaces and substrates, occupying spaces and consuming nutrients.

It limits the growth of pathogens, restricting their establishment in plants. Chitinases and Beta-1,3-glucanases produced by *Trichoderma* spp. are vital keys to its action against other fungi. The production of these enzymes is part of the process known as mycoparasitism, where, upon recognizing chemical signals from the cell wall of the pathogen, *Trichoderma* hyphae grow towards and wrap around the hyphae of the pathogenic fungus. They then initiate the secretion of enzymes that degrade and create pores in the cell wall, through which *Trichoderma* obtains nutrients [74,75].

Regarding indirect mechanisms, the activation of the plant's defense system is observed. This is because *Trichoderma* spp. emit chemical signals that stimulate the production of defensive compounds in plants, such as phytoalexins and enzymes [76,77].

Organic compounds, such as VOCs, are also produced by *Trichoderma* spp. and influence the germination of fungal spores, inhibiting their development. VOCs produced by *Trichoderma koningiopsis* T-51 inhibited the mycelial growth of *B. cinerea* by 73.78% and of *Fusarium oxysporum* by 43.68%. Additionally, there was a reduction in conidial germination and a delay in germ tube elongation [59].

It is important to highlight that the production of metabolites can vary between different species and strains of *Trichoderma*, as well as the environmental conditions. These metabolites play critical roles in the ecology of *Trichoderma* and their interactions with plants and other microorganisms, consolidating their role in controlling plant diseases and promoting soil health. Additionally, these fungi also play a vital role in improving nutrient and water uptake [78–80].

Out of 251 *Trichoderma* isolates from the Amazon rainforest soil, 49 demonstrated phosphate solubilization capacity. The production of organic acids, such as lactic acid, fumaric acid, ascorbic acid, malic acid, gluconic acid, D-isocitric acid, phytic acid, and citric acid, was observed. Additionally, two strains showed growth-promoting activity in soybean plants in a greenhouse setting [57]. High solubilization activity and growth promotion were also confirmed for *Trichoderma asperellum* UFT 201 in soybean cultivations in a greenhouse, and these results were later replicated in a field experiment [81,82].

The combination of these action mechanisms confers significant benefits to the plant. After selecting four strains with positive inhibitory activity against *F. oxysporum*, phosphate solubilization, and IAA production, an evaluation of tomato seed inoculation confirmed an increase in chlorophyll levels, aerial part length, fresh and dry weight of both the aerial part and roots, and a reduction in wilt disease caused by *F. oxysporum* ranging from 10 to 30% [83].

3.2. Arbuscular Mycorrhizal Fungi: Extensions of Roots in Soil

AMF have a symbiotic relationship with plant roots. Intracellular hyphae form arbuscules, specialized structures that penetrate the root epidermis to exchange nutrients with hosts [84]. AMF provide many benefits to plants, such as increased photosynthetic rates, improved soil quality, influence on atmospheric CO_2 fixation [85], enhanced nutrient absorption (particularly P), increased water uptake, protection against pathogens, and assistance in obtaining micro- and macronutrients [86].

Benefits arise from AMF functioning as extensions of roots, penetrating other parts of the rhizosphere. Additionally, their hyphae are thinner compared to roots, allowing them to access a greater soil volume. For protection against water deficit, AMF hyphae enhance access to small soil pockets [87]. Also, the presence of phytohormones like abscisic acid modulates plant mechanisms related to water deficit, such as aquaporins and transpiration [88]. AMF can also increase flood tolerance, improving growth and assisting in phosphorus absorption through osmotic adjustment performed by the fungi [89].

Heat stress is also attenuated by AMF, which increases nutrient and water absorption, along with improved photosynthetic rates. Other effects are the accumulation of proline and sugars, sodium reduction, increased carbon, and homeostasis [90]. These mechanisms, aiding in extreme temperature tolerance, also contribute to salinity tolerance [91]. Therefore, AMF plays a pivotal role in terrestrial ecosystems' microbiomes, helping host plants and ecosystem maintenance [92].

Among its roles in plant health regarding enhanced nutrient obtention, P absorption is the most thoroughly described and recognized as the primary regulator of the association. In conditions of high available concentrations of this mineral in the soil, the plant tends to reduce its investment in the association, whereas, under conditions of low availability, plant investment is higher. This enhanced P absorption occurs directly, through the absorption and transport of P via the hyphae to the arbuscules, and indirectly, by stimulating phosphate-solubilizing bacteria in the soil [93].

This stimulation occurs in the region known as the mycorrhizosphere, which is the zone around roots colonized by mycorrhizal fungi, directly influenced by hyphal and root exudation [94]. Composed of hyphae with a diameter of approximately 0.2 μm, much finer than roots but significantly denser, the mycorrhizosphere is broader than the rhizosphere and acts as a matrix, facilitating interactions between bacteria and fungi. Extraradical fungal hyphae form the hyphosphere, a region influenced by AMF hyphae, that bring about physical, chemical, and biological alterations. Examples of these alterations include soil particle aggregation and the direct delivery of water to the host plant [95,96].

Fungal hyphae release carbon-rich compounds promoting bacterial growth, and, on the other hand, hyphae can also produce compounds that signal or inhibit other microorganisms' growth [97], affect soil pH, maintain a liquid film, and serve as bacterial concentration nodes, creating a microbiome that supports soil maintenance [98].

Through the hyphal exudation of signaling molecules and compounds, AMF can influence the surrounding soil, modifying the local microbiota, referred to as the hyphosphere effect. It can also impact weathering, where exudates act as chelators, destabilizing mineral surfaces [99,100]. Thus, the hyphosphere provides an excellent habitat for other microorganisms, supporting the soil microbiome and influencing various soil processes [101].

4. Co-Inoculation of Beneficial Microorganisms

In recent years, there has been increased interest in combining microorganisms with similar activities but different mechanisms of action. This management strategy has been primarily studied in the control of diseases caused by nematodes, fungi, and insects, resulting in a reduction in the use of chemical products and a decreased selection of resistant pathogens. [102,103].

The interaction between growth-promoting microorganisms does not always result in the improvement in plant development or the biocontrol of pathogens; it is subject to several factors, such as the genetic variability of native bacteria, hosts, and environmental factors, such as light, temperature, and the organic matter of the soil [104].

These interactions are classified as synergistic, antagonistic, or non-interactive/additive, according to the observed effect. Synergy occurs when a combined effect is observed, meaning that co-inoculation provides a greater effect compared to the application of these agents individually. On the other hand, in antagonism, this effect is negative, whereas in non-interactive interactions, there is no impact compared to the isolated application of each microorganism [105].

An example of a synergistic interaction is the co-inoculation between AMF and PGPR, which has been examined by several studies, especially nitrogen-fixing bacteria (NFB). In general, gains in biomass and minerals were achieved, particularly in nitrogen and phosphorus rates [89,106].

The activity of AMF and NFB in the rhizosphere is essential for plant nutrition, and various factors contribute to a tripartite symbiosis. These microorganisms do not compete for the same colonization sites, indicating coexistence and possibly functional interactions. Furthermore, the inoculation of AMF has already been shown to be important in establishing NFB and improving nodulation, as nodulation is dependent on high levels of P, which can be increased by AMF colonization [104]. It is also worth noting the importance of the correct combination of strains to achieve synergy in the co-inoculation of these microorganisms [107] (Table 3).

Table 3. Benefits of inoculating microorganisms.

Species	Culture	Benefits	References
Bradyrhizobium diazoefficiens and *Rhizobium tropici*	Common beans (*Phaseolus vulgaris*)	Growth promotion and grain yield	[108]
Bradyrhizobium japonicum and *Azospirillum brasilense*	Soybean (*Glycine max*)	Increased yield components, grain yield, and seed quality	[109]
Pseudomonas fluorescens and *A. brasilense*	Tomato (*Solanum lycopersicum*)	Increased yield and fruit quality	[110]
Bradyrhizobium sp. and *Trichoderma* sp.	Cowpea (*Vigna unguiculata*)	Increased the growth rate, biomass, and photosynthetic pigments	[111]
Bacillus licheniformis and *Bacillus subtilis*	Cucumber (*Cucumber sativus*)	Alleviated salt stress	[112]
B. subitilis, *Bacillus megaterium* and *Rhizophagus intraradices*	Soybean (*G. max*)	Increase leaf nutrient and in yield	[113]
Rhizophagus irregulares and *Bradyrhizobium* sp.	Mung bean (*Vigna radiata*)	Growth promotion and alleviated water stress	[106]

5. Scenario of Inoculants Registered in Brazil

According to the Brazilian Ministry of Agriculture (MAPA), there are 636 inoculants with 713 registrations (some inoculants are registered for multiple crops) in Brazil (as of April 2024, based on the Ater digital platform provided by the Brazilian Federal Government). These registrations encompass 37 crops, ranging from vegetables like lettuce, cabbage, and potatoes, but primarily focusing on grains [114]. Approximately 50% of these registrations are for soybean cultivation, the most extensively planted crop in the country, covering an area of approximately 45 million hectares [115]. Bean and maize, also among the most cultivated crops in the country, have 73 and 70 registrations, respectively. Jack bean (35), peanut (36), and wheat (23) follow as the crops with the next highest number of inoculant registrations. The remaining registrations are distributed among the other 31 crops, with lettuce notably having eight (Figure 2).

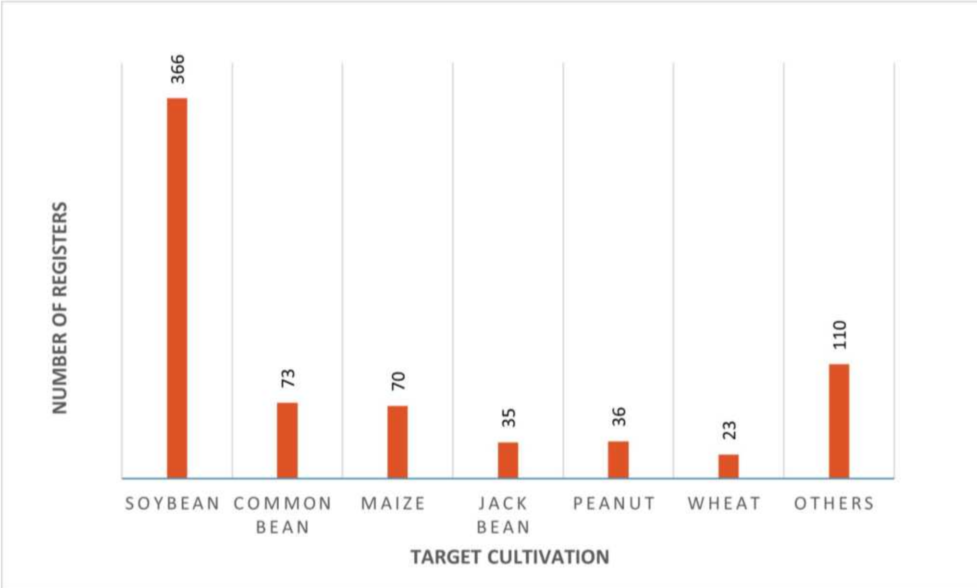

Figure 2. Number of inoculants registered for the main crops in Brazil.

5.1. Formulations: Single and Co-inoculation

Among these registrations, 622 consist of formulations with a single microorganism, with a significant portion being NFB such as *Bradyrhizobium* spp., *Azospirillum brasilense*, and *Rhizobium* spp. For soybeans, for example, there are 189 registrations of products with *Bradyrhizobium japonicum*, 57 for *Bradyrhizobium Elkani*, and 52 with the co-inoculation of *B. japonicum* and *B. elkani*. For beans, 65 out of 73 registrations are for *Rhizobium tropici* and 6 are for *A. brasilense*. In maize cultivation, 45 out of 70 registrations contain *A. brasilense* [114].

The introduction of these inoculants, commencing in 1964, played a crucial role in establishing Brazil's competitiveness in grain production and reducing the need for nitrogen fertilization. Recent years have seen the implementation of formulations co-inoculating two NFB strains, resulting in increased yields compared to individual inoculation. This co-inoculation strategy is expected to gain further traction in the coming years [116]. According to Telles et al. (2023), during the 2019/2020 harvest season, co-inoculation with *Bradyrhizobium* spp. and *A. brasilense* was employed in 25% of all soybean-cultivated areas in Brazil, yielding cost savings of 15.2 billion dollars by replacing urea application with biological nitrogen fixation. Additionally, it generated an estimated profit of 914 million dollars [117]. In the 2022/2023 harvest season, it is estimated that this practice was applied in 35% of soybean production in the country, a 10% increase compared to the previous season [6].

When analyzing a subset of the most important crops (Table 4), in addition to NFB, there are also registrations of plant growth-promoting rhizobacteria (PGPR) from the genus *Bacillus* and *Pseudomonas fluorescens*. In certain cases, co-inoculation between two strains is implemented, such as *B. subtilis* and *B. megaterium* for soybean, maize, and bean crops, *B. megaterium* and *Lysinobacillus* sp for maize, strains that promote growth by providing phosphorus availability, and *B. licheniformis* and *Bacillus aryabhattai* act on increasing stress tolerance in maize. The *B. megaterium* B119 and *B. subtilis* B2084 strains, registered as phosphate solubilizers, are multifunctional microorganisms that produce high levels of phytohormones, fix nitrogen, and produce siderophores. Additionally, they occupy distinct ecological niches, one being a rhizosphere isolate and the other an endophyte of maize roots, thus expanding the spectrum of action and specificity for maize cultivation, with gains in productivity and grain P content in low-fertility soils [118].

Table 4. Genus of microorganisms registered for the main crops in Brazil, organized in single inoculation or co-inoculation.

Culture	Genus	Single Inoculation	Co-Inoculation
Soybean (*Glycine max*)	*Bradyrhizobium* sp. *Azospirillum* sp. *Bacillus* sp. *Pseudomonas* sp. *Trichoderma* sp. *Rhizophagus* sp. (*Rhizoglomus*)	246 28 8 6 5 5	*Bradyrhizobium Japonicum* + *Bradyrhizobium Elkani* (52) *B. Japonicum* + *Azospirillum brasilense* (4) *A. brasilense* + *Pseudomonas fluorescense* (2) *Bacillus megaterium* + *Bacillus subtilis* (2) *B. subtilis* + *B. elkani* (3) *Rhizoglomus intraradices* + *Claroideoglomus claroideum* (2) *B. subtilis* + *B. elkani* +*Parabhurkodelia nodosa* (3) *B. subtilis* + *Bacillus amyloliquefacens* +*Bacillus pumilus* (1) *P. fluorescense* + *B. amyloliquefacens* + *Priestia megaterium* (1)
Common beans (*Phaseolus vulgaris*)	*Rhizobium* sp. *Azospirilum* sp. *Bacillus* sp.	65 6 1	*B. megaterium* + *B. subtillis* (1)
Maize (*Zea mays*)	*Azospirillum* sp. *Bacillus* sp. *Rhizophagus* sp. (*Rhizoglomus*) *Pseudomonas* sp. *Methylobacterium* sp.	41 8 5 3 1	*B. megaterium* + *B. subtilis* (2) *A. brasilense* + *P. fluorescense* (2) *B. megaterium* + *Lysinobacillus* sp. (1) *B. licheniformis* + *Bacillus aryabhattai* (2) *B. Japonicum* + *A. brasilense* (2) *R. intraradices* + *C. claroideum* (2) *B. subtilis* + *B. amyloliquefacens* +*B. pumilus* (1)
Peanut (*Arachis hypogaea*)	*Bradyrhizobium* sp.	36	-
Jack bean (*Canavalia ensiformis*)	*Bradyrhizobium* sp.	35	-
Wheat (*Triticum aestivum*)	*Azospirilum* sp.	23	-

In addition to *P. fluorescens* and *A. brasilense* for maize and soybean crops, there are also strains of *B. subtilis* and *B. elkani* for soybean. In these latter two cases, there are the co-inoculation of strains with different activities, biological nitrogen fixation, and phosphorus solubilization. This is an excellent alternative, given that some studies have already shown that a higher P content can influence the amount of N fixed in the soil [104,119]. The addition of K-solubilizing bacteria to these formulations could be a strategy used to increase the absorption efficiency of the three main macronutrients applied in Brazilian soils.

Co-inoculation with three strains is also observed, such as *B. subtilis*, *Bacillus amyloliquefaciens*, and *B. pumilus* for promoting growth in soybean and maize, and *P. fluorescense*, *B. amyloliquefaciens*, and *Priestia megaterium*, as well as *B. subtilis*, *B. elkani*, and *Parabhurkodelia nodosa* for soybean. This last species, introduced in the year 2024 to the market, isolated only in Brazilian soils so far, exhibits nitrogen fixation activity, among other beneficial interactions such as phosphate solubilization, hormone production, siderophore synthesis, and ACC deaminase activity [120].

In terms of fungi, the number of registrations is reduced to five registrations of *Trichoderma* for soybean and five registrations of AMF for soybean and maize. Among these, one formulation contains eight strains (4 *Trichoderma harzianum*, 3 *Trichoderma asperelloides*, and 1 *Trichoderma koningiopsis*), which are registered for soybean, alongside the other *Trichoderma* products. As for AMF, the registrations are for soybean and maize, containing a strain of *Rhizophagus intraradices* or a double inoculation with *R. intraradices* and *Claroideoglomus claroideum* (Table 4).

5.2. Biofertilization

Brazil consumes approximately 8% of the global fertilizer market, accounting for about 85% of the fertilizers applied domestically, with an annual expenditure of USD 25 billion. In 2020, domestic production of nitrogen fertilizers met only 4.3% of the national demand, a figure that has been declining due to the accelerated growth of the agricultural sector outpacing fertilizer production. In 2010, this figure was 20.7%. Regarding phosphorus (P) and potassium (K), imports accounted for 73% and 97%, respectively, highlighting a high external dependence that exposes Brazil's primary economic sector to fluctuations in the international fertilizer market. This situation is exacerbated when considering the utilization efficiency of these inputs, ranging from 50 to 70% for N, 15 to 50% for P, and 50 to 70% for K. This inefficiency is primarily attributed to the inadequate transfer of fertilization technologies designed for temperate climates to tropical regions, which experience higher rainfall, greater microbial activity, and highly weathered soils. In 2022, the Brazilian Federal Government introduced the National Fertilizer Plan (NFP), aimed at reducing the importation of these inputs from 85% to 45% by 2050. The development of more efficient biological inputs is one of the strategies outlined in the plan [4].

Brazil's inoculants market is experiencing substantial growth. NFB-based inoculants are well established in the production of various crops, most notably maize and soybeans. Nonetheless, the adoption of inoculants with alternative growth-promoting mechanisms remains in its early stages. Analogous to NFB in the context of nitrogen fertilization, AMF and PSB have the potential to revolutionize phosphate fertilization practices in Brazil. More than 70% of phosphorus fertilizers applied in Brazilian soils are imported, generating a variable cost according to the dollar exchange rate, and are poorly utilized. It is believed that, on average, only 30% of this phosphorus is absorbed by plants, with a significant portion of the surplus accumulating in the soil. Brazilian soils contain elevated concentrations of P in insoluble forms, a consequence of decades of fertilization in iron (Fe) and aluminum (Al)-rich soils, culminating in the rapid immobilization of this vital nutrient. It is estimated that by 2018, Brazil accumulated a staggering 33.4 teragrams (Tg) of phosphorus in its soil. This estimation is derived from an analysis of the ratio between phosphorus inputs from organic manure and mineral fertilizers and the phosphorus harvested by crops annually, dating back to 1967, a seminal year that marks the commencement of intensive phosphate fertilizer application. This accumulated phosphorus reservoir possesses an estimated economic value of USD 22 billion [121].

The inoculation of AMF and PSB is an important alternative to enhance the efficiency of P absorption, reducing the need for application and utilizing the soil's legacy P [121,122]. However, the application of these microorganisms is still not widely disseminated in Brazil. For example, AMF products available are imported and do not utilize native strains from the country, highlighting a gap in investment in technologies for strain research and the development of production systems for these microorganisms. Cely et al. (2016) demonstrated the ability of the arbuscular mycorrhizal fungus (AMF) *R. clarus*, cultivated in vitro in association with transformed carrot roots, to enhance the effectiveness of P application in soybean and reduce the required dose by half in cotton under field conditions. This cultivation method is efficient for producing large quantities of propagules, free from contaminants and with homogenous batches, but still requires scaling up [62].

In the case of PSB, although products are already available on the market, they have not yet achieved widespread adoption. However, the trend indicates an increase in product availability, with the registration of new species and a rise in the number of doses applied in upcoming harvests. Mosela et al. (2022) confirmed the high capacity of the *Bacillus velezensis* strain Ag 75 to improve P application efficiency in soybean and maize crops under field conditions, a species not yet commercially available [15].

Like phosphate fertilizers, potassium fertilizers are largely imported, around 97%, a percentage that has been increasing in recent years due to the accelerated growth of agriculture [115]. It is estimated that the efficiency of applied potassium (K) in the soil is

66%, with a considerable portion remaining stocked up in the soil and 13% lost through erosion and leaching processes [123].

Brazil is a country endowed with extensive mineral resources, among which potassium-containing rocks hold potential as a nutrient source for agriculture. Currently, many rock-derived powder products are registered as remineralizers by MAPA (the Brazilian Ministry of Agriculture). Among these, some are primarily composed of glauconite, a mineral found in rocks such as greensand, slate, and glauconitic siltstone, serving as a significant source of K [124]. However, the availability of potassium from these sources depends on the physicochemical conditions of the soil; generally, only 1 to 2% is directly available for plant uptake, with the remainder existing in non-exchangeable forms in the soil [123]. To enhance potassium availability, investments are needed in research and development of strategies to facilitate the rapid release of these nutrients for plant absorption. These processes can be thermal, chemical, or biological in nature [124,125].

Research based on biological solubilizers is still limited but demonstrates potential and should be further explored, as these methodologies tend to be less costly compared to thermal and chemical processes. In a study utilizing greensand as a K source, *Burkholderia* sp. and *Bacillus* sp. strains were able to extract 71.3% and 53.6% of K, respectively, compared to the control [125]. Matias et al. (2019) demonstrated the ability of the bacterium *Acidithiobacillus thiooxidans* to solubilize K from greensand, primarily through medium acidification, from pH 4.2 to 0.57 after 49 days of incubation [126].

According to the National Fertilizer Program (NFP), by 2050, Brazil intends to be a reference in the significant production of remineralizers and other alternative sources of potassium derived from silicate rocks. Exploring strains with high potassium solubilization activity and developing biological products that enhance the availability of this nutrient represent gaps to be addressed in the Brazilian market [5].

5.3. Abiotic Stress Mitigation

Other inoculants that are expected to see increased registrations in the coming years are stress mitigators. The effects of adverse weather conditions are already being felt in Brazilian agriculture. According to the National Supply Company (Conab), the estimate for the harvest in the 2023/2024 season is 8% lower compared to the previous season, a reduction of around 25.7 million tons. This loss of productivity is directly related to the delayed onset of rains in the Midwest, Southeast, and Matopiba regions, high temperatures, irregular and poorly distributed rainfall, and periods of drought lasting more than 20 days. In addition to the impacts on productivity, these effects may, in the long term, affect the cultivable regions for Brazil's main crops, reducing areas suitable for soybean and maize cultivation [127]. Currently, there are already *Bacillus* sp. strains on the Brazilian market for reducing water stress, registered for soybean and maize crops. The CMAA1363 strain of *B. aryabhattai* was prospected from cactus rhizospheric soil in the Caatinga biome of the Brazilian semiarid region during the dry season [128]. Evaluations in four different edaphoclimatic regions confirmed that this *B. aryabhattai* strain is a potent growth promoter, increasing maize yields by 5.9 to 43.7% [129]. In a study conducted on maize in an agroecological system, inoculation with the CMAA1363 strain partially mitigated the impacts of water and salt stress through morphophysiological characteristics, such as increased leaf area and plant height [130].

However, given the magnitude of the economic impact that these climate changes can cause, there is a trend towards increased investment in research and development of biological products to mitigate the effects of climate change on agriculture and a greater variety of strains and formulations covering more cultures. The impact of AMF inoculation on plants under abiotic stress is well documented in the literature. In addition to their role in nutrient absorption, these microorganisms have high potential as multifunctional inoculants and for co-inoculation with PGPB [131,132].

6. Future Perspectives

The application of inoculants has been growing worldwide. With increasing research on the impact of chemical inputs on the environment and human health, and the recognition of the importance of microorganisms in nutrient cycling and maintaining soil quality, production systems are undergoing a transition towards more sustainable agriculture. The use of these inputs is among the strategies used to achieve the sustainable development goals set by the United Nations General Assembly and is seen as essential for maintaining global food security [133]. As one of the largest agricultural producers globally, Brazil has been rapidly adapting its legislation and accelerating the registration process for biological products. With the collaboration of companies, research institutes, and the consumer market, processes and parameters are being defined to ensure product quality, from research for registration to final product quality control. Given the emergence of new technologies, this regulatory process must be continuous in the coming years.

In this context, research on the impact of PGPBs on various crops has been increasing. It is likely that more specific inoculants will be registered, tailored to the needs of each crop. With the aid of precision agriculture, the application of these bio-inputs can be targeted based on the characteristics of the region, soil, and plant, optimizing their utilization.

In the case of Brazil, the application of inoculants will be essential for reducing external dependence on fertilizers and improving the utilization of nutrients retained in the soil. Investment in research on P-solubilizing microorganisms is necessary, as they are still not well established in the market but have great potential for growth in the number of registered products and are expected to have more doses applied in the coming harvests. It is also crucial to encourage research on K-solubilizing bacteria, which currently have limited studies, as they may hold the key to utilizing alternative sources of this nutrient. The application of inoculants will also be a vital tool for mitigating the impact of climate change. To achieve this, research should focus on increasingly efficient strains and more resistant formulations that extend shelf life and maintain the performance of microorganisms in the field, even under adverse environmental conditions.

The utilization of new biotechnologies will be of paramount importance for the development of higher-quality, more complex bio-inputs in a shorter timeframe. Advances in sequencing technology will aid in the identification and prospecting of improved strains. Through mining the genomes of competent strains, we can identify and reveal the functions of genes involved in specific mechanisms. More research is also needed to understand the interaction between microorganisms and plants in the soil, identifying beneficial metabolites and compounds produced by growth-promoting microorganisms. Based on these data, genetic engineering can be employed to optimize the activity of strains, but the application of products with modified microorganisms requires debate regarding their impact on soil ecology, ethical considerations, and regulatory frameworks [134].

Regarding the extension of shelf life, new formulations of agricultural inoculants have evolved significantly, incorporating carriers and encapsulation technologies that enhance the efficacy and stability of beneficial microorganisms. Innovative carriers, such as biodegradable polymers and biomass matrices, provide an ideal environment for the survival of inoculants, ensuring controlled and prolonged release. Encapsulation, in turn, protects microorganisms from adverse conditions like heat and desiccation and enables targeted release in the soil, optimizing root colonization. These innovations not only improve the viability of inoculants but also enhance agronomic benefits, such as increased productivity and crop sustainability [134].

7. Conclusions

The utilization of PGPR and PGPF as biological inoculants has proven to be efficient and capable of generating economic benefits for agricultural production systems, as evidenced by the individual or co-inoculated use of *Bradyrhizobium* spp. and *A. brasilense*, contributing to the sustainability of Brazilian agriculture. With the well-established use of NFB in the country, the focus in the coming years should be on establishing and scaling up the application of PSB, along with the registration of new strains and more efficient formulations. The development of AMF-based products is also crucial, as their multifunctionality makes them valuable tools for agriculture, contributing to nutrient availability, mitigating the impact of abiotic stress, and offering synergistic potential in co-inoculation with PGPB. Additionally, investment in research is needed to expand our understanding of the potential of K-solubilizing bacteria in extracting this nutrient from alternative sources, which can help reduce external dependence on chemical fertilizers.

Climate change is expected to be one of the major challenges of the coming decades and could drastically reduce Brazilian agricultural cultivation areas and production. This problem requires constant monitoring, research, and development to mitigate its effects on agriculture. The selection of improved strains and the development of new inoculant formulations with extended shelf life and enhanced field performance will be crucial in addressing the challenges posed by climate change and ensuring sustainable agricultural practices.

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EDITED BY
Ashwani Kumar,
University of Allahabad, India

REVIEWED BY
Geison Souza Izídio,
Federal University of Santa Catarina, Brazil
Rajeshwari Negi,
Eternal University, India
Rosario Razo Belman,
Universidad de Guanajuato, Mexico

*CORRESPONDENCE
Leandro Simões Azeredo Gonçalves
leandrosag@uel.br

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The current increase and future perspectives of the microbial pesticides market in agriculture: the Brazilian example

Matheus Felipe de Lima Andreata[1], Silas Mian[2,3], Galdino Andrade[1], Adeney de Freitas Bueno[4], Mauricio Ursi Ventura[2], José Eduardo Marcondes de Almeida[5], Eduardo Augusto Fonseca Ivan[6], Mirela Mosela[1,3], Ane Stéfano Simionato[3], Renata Rodrigues Robaina[3] and Leandro Simões Azeredo Gonçalves[2]*

[1]Microbiology Department, Universidade Estadual de Londrina (UEL), Londrina, Brazil, [2]Agronomy Department, Universidade Estadual de Londrina (UEL), Londrina, Brazil, [3]BIOINPUT Research Company, Cambé, Brazil, [4]Embrapa Soja, Londrina, Brazil, [5]Instituto Biológico de São Paulo, São Paulo, Brazil, [6]BRANDT Brasil, Cambé, Brazil

The Brazilian agricultural sector contributes 25% to the national gross domestic product (GDP) and accounts for 49% of the country's exports, faces significant challenges associated with tropical agriculture. Pests and diseases are major issues that compromise the productivity of various crops. In response, microbial pesticides have increasingly been incorporated as a component of integrated pest and disease management (IPM and IDM, respectively). This study presents a comprehensive analysis of the Brazilian biopesticide market, focusing on bioinsecticides, bionematicides, and biofungicides. Microbial agents, such as *Bacillus* spp., *Trichoderma* spp., and *Beauveria* spp., play a important role in IPM and IDM strategies, acting through multiple biocontrol mechanisms. The biopesticide market in Brazil has grown rapidly, driven by increased adoption by farmers and recent regulatory advances that have facilitated these products' registration and commercialization process. Projections indicate that this sector will continue to grow in the coming years, supported by research innovations, consolidating biopesticides as key elements in Brazil's transition to more sustainable agriculture. This review explores the challenges, opportunities, and future trends of microbial pesticides in Brazilian agriculture, highlighting their potential in increasing crop resilience and productivity while reducing the environmental impact associated with conventional pesticides.

KEYWORDS

biological control, biofungicides, bioinsecticides, bionematicides, regenerative agriculture

1 Introduction

Sustainable intensification of agriculture is crucial for global food security, and its population is expected to reach 9.6 billion by 2050 (Tripathi et al., 2019). Brazil is highlighted as a major agricultural player among the most important countries where intensive agriculture is required to advancing this important agenda. Therefore, agribusiness is an important sector of the Brazilian economy, accounting for ¼ of the country's gross domestic product (GDP) and 49.3% (US$165.55 billion) of exports in 2023. Approximately 40.4% of the foreign revenue in this sector comes from soybeans and their byproducts (grain, meal, and oil). In addition to being the world's largest exporter and producer of soybeans, Brazil is also considered a leading global producer of other economically important crops, such as sugarcane, corn, coffee, cotton, beans, oranges, and bananas.[1] Despite the positive agricultural outlook for these crops, significant concerns have arisen due to biotic and abiotic stress, leading to considerable losses in yield in terms of amount and quality.

Global productivity losses caused by pests and crop diseases threaten economies and food security, particularly in developing countries (Ristaino et al., 2021; Singh et al., 2023). It is estimated that, on average, these losses are 21.5% (10.1–28.1%) for wheat, 30.3% (24.6–40.9%) for rice, 22.5% (19.5–41.1%) for maize, 17.2% (8.1–21%) for potatoes, and 21.4% (11–32.4%) for soybeans (Savary et al., 2019). However, the most significant losses are observed in regions with food deficits, rapid population growth, and emerging and reemerging pests and diseases (Savary et al., 2019). The estimated annual cost to the global economy due to losses from pests and diseases exceeds US$220 billion (FAO, 2022).

Over the past few decades, chemical control has become important in mitigating crop losses caused by pests and diseases (Ansari et al., 2014). Despite advancements in alternative strategies, crop protection remains the predominant approach among farmers (Serrão et al., 2022). However, the heavy reliance on traditional chemicals in agriculture poses risks to human health and the environment (Gagic et al., 2021; Jacquet et al., 2022). In a systematic review, Lopes-Ferreira et al. (2022) reported that occupational exposure to pesticides in Brazil is associated with several adverse health effects in farm workers, including hematological, endocrine, neurological disorders, and cancer. This situation is intensified by permissive regulations that allow the use of substances banned in other countries and reclassify highly toxic compounds into less restrictive categories (Pereira et al., 2022; Souza et al., 2023).

In addition to human health concerns, chemical pesticides are also associated with the reduction of natural biological control agentes (Torres and Bueno, 2018) and pollinators (dos Santos et al., 2018), while driving the evolution of pest and disease resistance (Serrão et al., 2022; Gong et al., 2023). Consequently, reducing the use of synthetic chemicals in agriculture to mitigate their adverse effects and pursuing more sustainable management practices (Maciel and de Freitas Bueno, 2023) have become global goals and government policies in various countries worldwide (Lee et al., 2019).

Additionally, managing plant pests and controlling diseases are significant components of agricultural production costs. Global spending on chemical control in agriculture is expected to increase from US$50.62 billion in 2017 to US$68.82 billion by the end of 2025 (Gagic et al., 2021). In this context, adopting integrated control measures such as integrated pest management (IPM) and integrated disease management (IDM) is considered an important strategy for enhancing control effectiveness (Dara, 2019; Abbas et al., 2022; Richard et al., 2022). IPM and IDM are sustainable decision-based approaches that integrate cultural, biological, behavioral, genetic, and chemical tools to identify, manage, and mitigate pest and disease risks. Biological control has emerged as a key strategy because of its effectiveness, minimal environmental impact, and direct benefits to plants, including enhanced growth and the activation of both local and systemic resistance (Collinge et al., 2022). Moreover, the development of commercial biological products is on average 75 times more cost-effective than that of synthetic chemical pesticides (Ram et al., 2018).

Therefore, the use of biological control measures has increased worldwide. More than 15% annual growth in the global biological control market had been recorded since reaching a value of US$3.3 billion in 2017 (van Lenteren et al., 2021) and approximately US$14 billion in 2023.[2] Projections indicate accelerated expansion of this market, which could reach US$49 billion by 2032. Biological control products in the market increased by 71.6% in a few years in Brazil alone. It went from 225 products in 2019 to 386 at the end of 2021 (De Bueno et al., 2023b). Data from Blink Consultancy[3] indicate that Brazil's biopesticide market reached US$690 million in the 2023/24 season, accounting for approximately 6% of the total agricultural pesticide market in the country. Among the product categories, bioinsecticides constituted 42.5% of the biopesticide sector, followed by bionematicides (30%) and biofungicides (27.5%). Market projections suggest sustained expansion in these segments, with the sector potentially doubling in size within the next 2–4 years, surpassing US$1.69 billion annually by 2027 and reaching an estimated US$2.91 billion by 2030 (Figure 1) (Borsari and Vieira, 2022). By the end of 2024, the Ministry of Agriculture, Livestock, and Supply (MAPA) had registered 309 products as bioinsecticides, 119 as biofungicides, and 96 as bionematicides,[4] illustrating the importance of Brazilian agriculture as a case study for adoption of biological control.

Consequently, we analyzed Brazil's microbial pesticide market in this study by examining the status of product registrations in MAPA and recent trends. It also projects the sector's trajectory within Brazilian agriculture, identifying the key drivers, challenges, and opportunities that may influence its development in the coming years.

2 Microbial biopesticides—mode of action

Biopesticides derived from microorganisms such as bacteria, fungi, viruses, and entomopathogenic nematodes are increasingly recognized for their effectiveness in controlling pests and diseases (Fenibo et al., 2021; Marrone, 2024). Biological control agents (BCAs) play an important role in both IPM (De Bueno et al., 2023a) and IDM (Ayilara et al., 2023) and can be applied either alone or in combination with synthetic pesticides (Maciel et al., 2024). This approach enhances control efficacy and reduces environmental risks and farmers' and consumers' exposure to synthetic pesticides. Moreover, microbial biopesticides help manage pest and pathogen resistance to chemical

1 http://www.ibge.gov.br

2 https://www.marketsandmarkets.com

3 https://blinkstrategies.com

4 https://agrofit.agricultura.gov.br

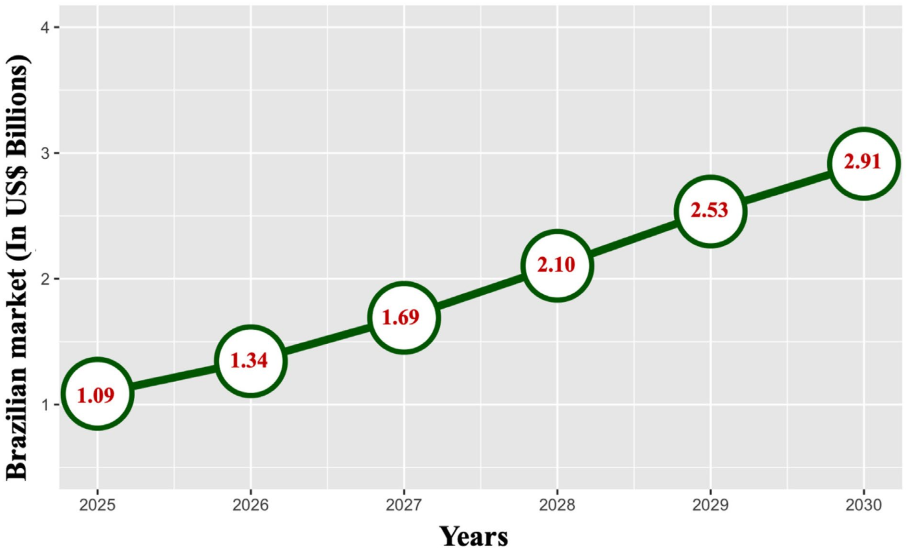

FIGURE 1
Projection of the Brazilian market for biologicals in pest control. (Exchange rate: US$ 1.00 = R$ 5.80.) Source: Adapted from Borsari and Vieira (2022) copyright Embrapa, 2022.

pesticides, a major issue resulting from the prolonged use of synthetic products (Ayilara et al., 2023; Koul, 2023).

BCAs can control pests and diseases, either directly or indirectly (Figure 2). These microorganisms directly produce secondary metabolites, including specific toxins that disrupt essential cellular processes such as protein synthesis and cell membrane integrity (do Nascimento et al., 2022; Marrone, 2024). Additionally, many BCAs produce lytic enzymes, such as chitinases and cellulases, which degrade the essential structural components of various pests and pathogens, leading to the breakdown of their physical defenses (Poria et al., 2021; Boro et al., 2022; Pandit et al., 2022). Mycoparasitism is another strategy by which beneficial fungi directly parasitize pathogens and destroy their cellular structures (Zaki et al., 2020; Dou et al., 2022). Disruption of quorum sensing, which prevents chemical communication between pathogenic cells, is important in preventing these cells from coordinating virulent attacks on plants (Maddela et al., 2020). Another BCA mode of action is microbial predation, in which predatory microbial species capture, ingest, and digest pathogenic microorganisms or pests. This process is a natural biological mechanism by which predatory microorganisms, such as certain bacteria, fungi, or protozoa, attack and consume pathogenic microorganisms or pests (Köhl et al., 2019; Vero et al., 2023). Other mechanisms that reduce pathogen virulence and competition for space and nutrients, such as hypovirulence induced by mycoviruses, are essential for limiting the growth of pathogenic microorganisms (Vero et al., 2023).

RNA interference (RNAi) has emerged as a promising and specific strategy for managing pests and pathogens in agriculture. By enabling the silencing of essential genes in target organisms through the application or in planta expression of double-stranded RNAs (dsRNAs), RNAi leads to gene knockdown that can impair development, reproduction, or even cause mortality (Adeyinka et al., 2020; Rodríguez Melo et al., 2023).

As an indirect defense mechanism in plants, BCAs can induce systemic resistance and enhance resilience to biotic and abiotic stress (Lahlali et al., 2022). These mechanisms include the activation of complex signaling networks involving phytohormones, such as salicylic acid, jasmonic acid, and ethylene, which are essential for defense against pathogens and pests, as well as for mediating plant-microorganism interactions (Elnahal et al., 2022; El-Saadony et al., 2022; Abdelaziz et al., 2023). BCAs such as *Bacillus* spp. and *Trichoderma* spp. induce systemic acquired resistance (SAR), priming plants with a rapid and robust immune response against a broad range of pathogens (Choudhary and Johri, 2009; Salwan et al., 2022; Rabari et al., 2023). Additionally, BCAs modulate the expression of defense-related genes, including those encoding enzymes such as chitinases and pathogenesis-related proteins (PR proteins), and facilitate the accumulation of biochemical compounds that restrict pathogen spread. Moreover, BCAs with ACC deaminase activity prevent ethylene-induced premature senescence, while promoting plant growth by producing growth regulators and improving nutrient availability (Lahlali et al., 2022). These complex and interconnected mechanisms demonstrate the effectiveness of microbial biopesticides in inducing broad-spectrum resistance and underscore their importance in regenerative agriculture.

3 Biopesticides market in Brazil

The biopesticide market has grown remarkably in Brazil in recent years, following the global trend. As previously mentioned, the sector's revenue, below US$58 million in 2017, reached US$690 million in the 2023/24 season, reflecting a compound annual growth rate (CAGR) of 49.38% (Figure 3A). When analyzed by segment, the CAGRs were 38.72, 66.38, and 63.65% for insecticides, nematicides, and fungicides, respectively, demonstrating the expansion and increasing adoption of these technologies in Brazilian agribusiness.

Data from MAPA (See text footnote 4) indicate that Brazil had 77 registered biocontrol products from 36 companies in 2016 (Figure 3B).

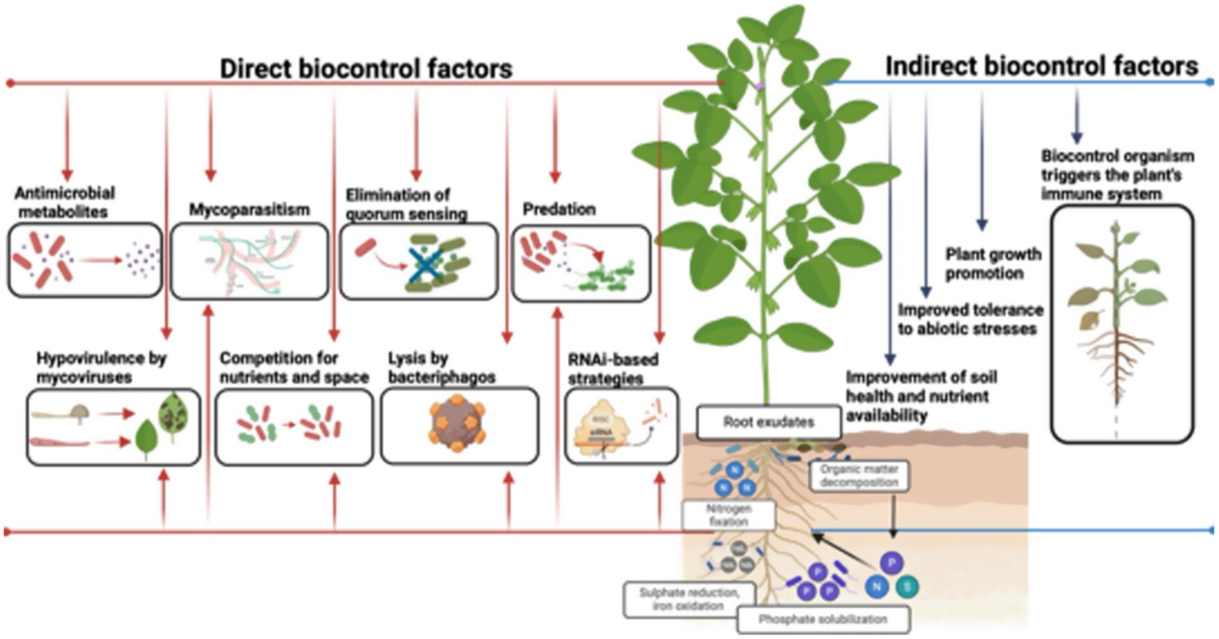

FIGURE 2
Mechanisms of action of microbial biopesticides in disease and pest management. This figure illustrates the complex interactions and pathways through which microbial biopesticides affect plants, including antagonism, competition, induced systemic resistance, and the key mechanism of direct pathogen inhibition. Additionally, it highlights the role of root exudates in shaping the rhizosphere microbiome and enhancing nutrient cycling processes, such as nitrogen fixation, phosphate solubilization, and organic matter decomposition. Source: Figure created using BioRender.com.

By 2020, this number more than tripled, reaching 236 registrations from 59 companies. As of December 2024, the total number of products rose to 515 across 101 companies. Although many companies focus on specific biocontrol categories, portfolio diversification trends exist. Despite the market's fragmentation and predominance of small national companies, the sector is steadily maturing. Currently, Brazil's biocontrol market provides a diverse array of products that target major agricultural pests and diseases (Figure 3C).

Economic, environmental, regulatory, and technological factors drive the expansion of Brazil's biopesticide market. Establishing a legal framework for bioinputs has played a role in streamlining the registration and commercialization of biological products, fostering an environment conducive to innovation and market growth. At the same time, support from government policies to support sustainable agriculture, together with research advances led by public and private institutions, has been fundamental to the consolidation of this technology. The Brazilian industry has also undergone professionalization to enhance the quality and efficacy of biocontrol agents. The entry of new companies and the diversification of product portfolios further reflect the sector's growing maturity and resilience.

3.1 Brazilian legislation

Brazil has improved its regulatory framework for biological products in alignment with expanding its biopesticide market. The legal landscape governing bioinputs has undergone transformations marked by key legislative milestones.

- Pesticide Law (1989): The first legal framework regulating agricultural inputs, this law broadly classified "products and agents of physical, chemical, or biological processes" but lacked precise definitions for BCAs. This ambiguity posed regulatory challenges for bioinputs.
- Decree No. 4074/2002: An advancement that formally defined BCAs and established precise registration requirements, including taxonomic classification, labeling, concentration, stability, and mode of action. In the same year, Agência Nacional de Vigilância Sanitária (ANVISA) introduced toxicity assessment criteria for vertebrates requiring acute, chronic, mutagenic, carcinogenic, and reproductive toxicity evaluations.
- Joint Normative Instructions (2005–2006, updated in 2014): As the sector grew, the regulatory framework expanded to include microbiological, biochemical, semiochemical, and macrobiological products through joint instructions issued by MAPA, IBAMA, and ANVISA. Under this framework, ANVISA assesses human toxicity; IBAMA evaluates environmental impacts, including risks to non-target species; and MAPA determines field efficacy.
- Joint Ordinance SDA/MAPA, IBAMA, and ANVISA No. 1/2023: Published on April 4, 2023, this ordinance replaced Joint Normative Instruction No. 3/2006, introducing updated registration procedures for microbiological products used in pest and disease control. It also redefines microbiological products to include both active microorganisms and their metabolites.
- Law No. 14785/2023: Repeated the 1989 Pesticide Law, removing outdated classifications and setting the foundation for a more specialized regulatory framework for bioinputs.

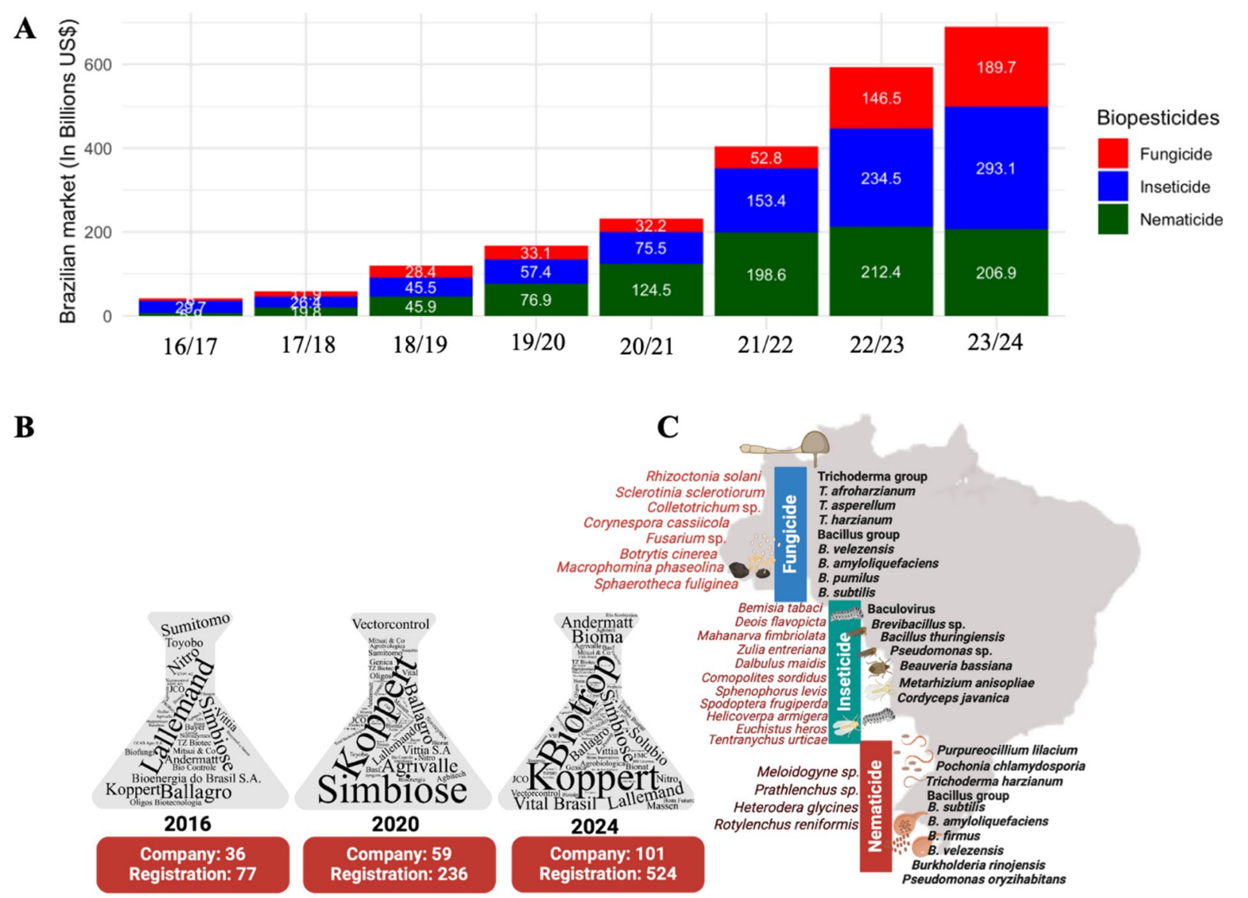

FIGURE 3
Market dynamics, key targets, and microbial species applied to Brazil's biocontrol of diseases and pests. **(A)** Growth of the biopesticide market in Brazil over the years, highlighting the number of microbial insecticide, nematicide, and fungicide registrations. **(B)** The distribution of companies and the number of microbial biopesticide registrations underscore the sector's diversity and representativeness and showcase the industry's breadth and depth. **(C)** Major target diseases and pests, and the predominant microbial species used as biopesticides. (Exchange rate: US$1.00 = R$5.80.).

- Law No. 15.070/2024: Established a dedicated regulatory framework for bioinputs, removing biological products from the scope of pesticide and fertilizer regulations. Key provisions include a single registration system for multifunctional products, simplified approval procedures for similar bioinput products, and authorization for active ingredient registration for on-farm production. The law exempts on-farm bioinput production from registration, provided that commercial sales remain prohibited. This practice must utilize strains sourced from accredited germplasm banks or registered products approved for this purpose. In addition, the law permits the production and transport of bioinputs among cooperative members and associations, ensuring compliance with good manufacturing practices and supervision by qualified technical professionals.

3.2 Microbiological insecticides

The number of registered microbial insecticides in Brazil has grown exponentially since 2014 (Figure 4A; Supplementary Table S1). The number of registrations remained extremely low between 2001 and 2013, with fewer than five new registrations annually. However, starting in 2014, there was a significant acceleration with a steady increase in registrations and target pests. This growth can be attributed to regulatory, technological, and marketing factors. The introduction of *Helicoverpa armigera* in Brazil and the occurrence of major outbreaks of this pest in 2013, resulting from unbalanced agriculture that relied exclusively and excessively on chemical control at the time, also reignited interest in IPM and the adoption of biological control by Brazilian farmers (de Freitas Bueno and Sosa-Gómez, 2014).

The introduction of the reference strains *Beauveria bassiana* IBCB 66 and *Metarhizium anisopliae* IBCB 425 by the MAPA in 2007 streamlined the biopesticide registration process. By establishing reliable and widely accepted standards, this measure significantly reduced regulatory complexity and production costs, particularly benefiting small and medium-sized companies. Consequently, it has facilitated market entry for new companies and accelerated the commercialization of biopesticides (Mascarin et al., 2024; McGuire and Northfield, 2020; Mesquita et al., 2023). Currently, 80 and 96% of *B. bassiana* and *M. anisopliae* registrations in Brazil, respectively, are linked to these reference strains. Beyond regulatory advancements, capacity-building initiatives have become instrumental in strengthening the sector. Research institutions such as the Biological Institute, Brazilian Agricultural Research Corporation (EMBRAPA),

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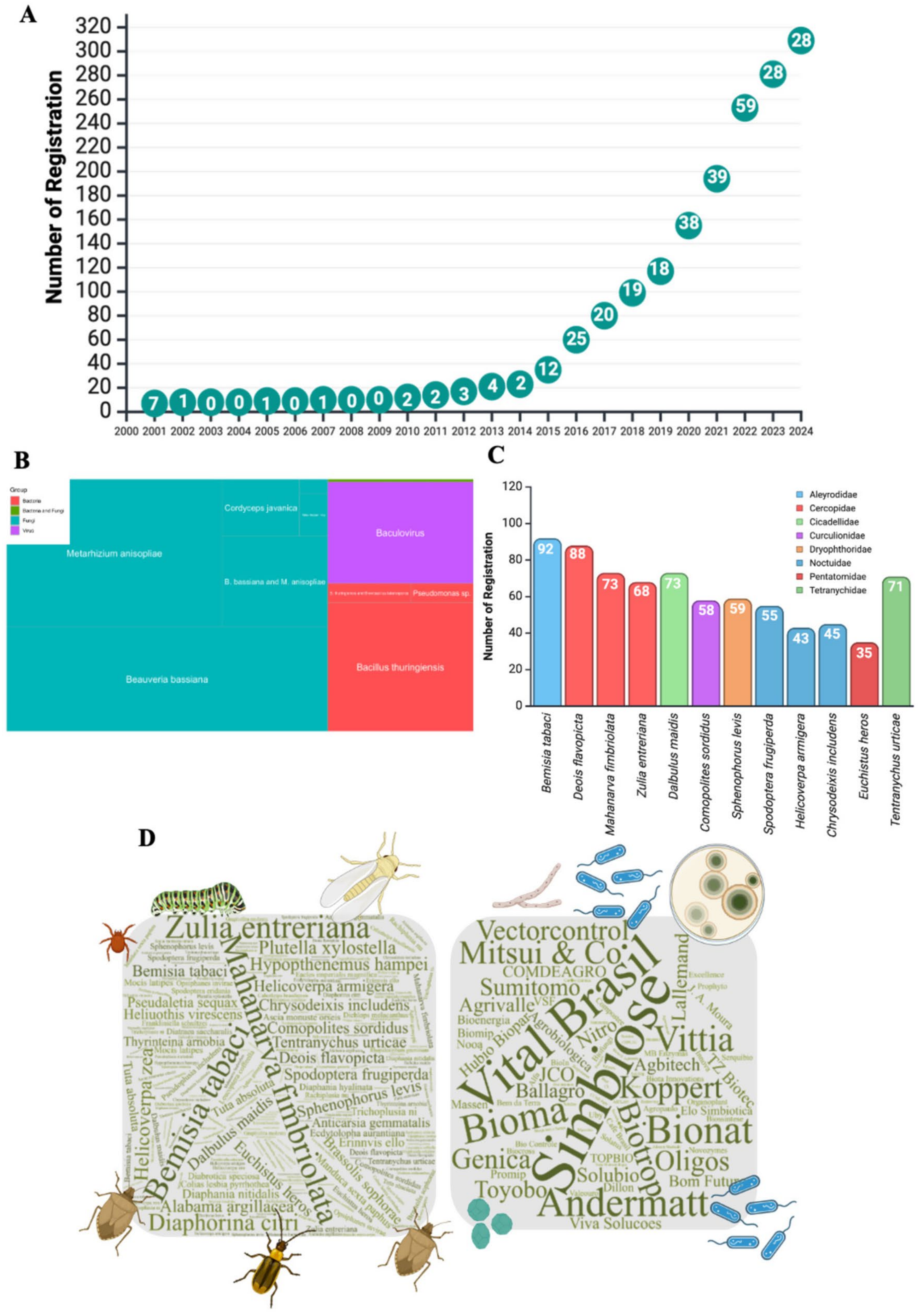

FIGURE 4
Registrations of microbial insecticide in Brazil: Market trends, key pathogens, and microbial species. **(A)** Annual trend in the number of microbial insecticide registrations in Brazil, reflecting the growing adoption of biocontrol agents. **(B)** The treemap visualizes the predominant microbial species utilized for pest biocontrol, categorized by organism type (bacteria, fungi, or viruses). **(C)** Registration counts for the most targeted pests, showing the relative focus on different pest species. **(D)** Word cloud illustrating the pests with the highest number of microbiological insecticide registrations and the leading companies involved in their registration in Brazil, highlighting the industry scenario.

and public universities have developed specialized training programs focused on the quality control and production of entomopathogenic fungi via solid fermentation. These programs have ensured that companies adopt rigorous production standards, enhance the quality and efficacy of biopesticides, and drive broader adoption in the field.

Research conducted by the "Luiz de Queiroz" College of Agriculture (ESALQ-USP) has been fundamental in driving innovation and developing microbial insecticides in Brazil. This institution is recognized for its studies on entomopathogenic fungi, such as *B. bassiana*, *M. anisopliae*, and *Cordyceps javanica* (formerly known as *Isaria fumosorosea*). Notably, most *C. javanica* registrations in Brazil were derived from strains developed by ESALQ, including ESALQ-1296, ESALQ-3422, and ESALQ-4778. In addition, SparcBio,[5] an innovation platform that connects academic research to the production sector, has played an important role in accelerating the development of new biological products. This platform aims to facilitate technology transfer, allowing scientific advances generated in academia to be quickly translated into commercial applications, thereby significantly strengthening Brazil's biocontrol market.

The entomopathogenic fungi *B. bassiana*, *M. anisopliae*, and *C. javanica* accounted for 68% of the microbial insecticide registrations in Brazil (Figure 4B), with *B. bassiana* and *M. anisopliae* being the most prominent with 91 and 83 registrations, respectively, in addition to 22 products that combined both species. *B. bassiana* is widely used to control pests such as the whitefly (*Bemisia tabaci*), sugarcane weevil (*Sphenophorus levis*), brown stink bug (*Euschistus heros*), and pasture and corn spittlebugs (*Deois flavopicta* and *Dalbulus maidis*). *M. anisopliae* is especially effective in controlling spittlebugs such as *Mahanarva fimbriolata*, *Deois flavopicta*, and *Zulia entreriana*. Additionally, two products based on *M. rileyi* targeted fall armyworms (*Spodoptera frugiperda*) (Mesquita et al., 2023).

Despite the continued growth of the market for entomopathogenic fungi, most commercially available products are based on wettable powder (WP) formulations (71%). The primary production technique for these fungi involves solid-state fermentation on rice substrates to achieve high yields of aerial conidia. However, little emphasis has been placed on developing formulations that extend the shelf life and improve pathogenicity. There is a widespread perception that companies lack investment and interest in formulation research to enhance the efficacy of these bioinsecticides, despite the importance of such improvements being widely highlighted in scientific literature (Mwamburi, 2016; Biryol et al., 2021; Meirelles et al., 2023; Mesquita et al., 2023).

Bacillus thuringiensis (*Bt*) has become a cornerstone of biological pest control in Brazilian agriculture, particularly following the widespread outbreak of *Helicoverpa armigera*, which caused severe damage to multiple crops, including maize, soybean, cotton (Czepak et al., 2013). Early attempts to manage this pest using conventional insecticides with diverse modes of action have proven ineffective. As an emergency response, Brazil imported baculovirus-based biopesticides from countries such as Australia and, alongside *Bt*, secured expedited registration with the MAPA for pest control (de Freitas Bueno and Sosa-Gómez, 2014). This has facilitated the rapid deployment of biological control programs, in which the combined use of baculovirus and *Bt* demonstrated high efficacy in suppressing *H. armigera*, driving significant growth in the Brazilian biopesticide market (Polanczyk et al., 2017; do Nascimento et al., 2022).

Bt-based bioinsecticides account for 17% of microbial insecticide registrations in Brazil. They are primarily used against key agricultural pests, including *Alabama argillacea*, *Chrysodeixis includens*, *Spodoptera frugiperda*, *Ecdytolopha aurantiana*, *Anticarsia gemmatalis*, and *H. armigera*. Among the registered products, the HD-1 strain dominated, representing 51% of approvals, with concentrated suspensions (SC) and WP comprising 61 and 22% of the formulations, respectively.

In response to evolving pest pressures, new bioinsecticide registrations have introduced formulations that combine *Bt* with *Brevibacillus laterosporus*, broadening their spectrum of action and enhancing their appeal to farmers facing simultaneous infestations. This combination offers a dual mode of action: while *Bt* produces insecticidal toxins, *B. laterosporus* actively colonizes the pest's digestive tract, proliferates, and induces septicemia, further increasing biocontrol efficiency (Smirnova et al., 2023).

Baculoviruses are also recognized as important biocontrol agents, especially for managing pests such as *S. frugiperda*, *A. gemmatalis*, and *H. armigera*, representing 6.7% of the microbial insecticide registrations in Brazil. Their integration into IPM programs has contributed to a reduction in reliance on chemical insecticides. One of the most notable advantages of baculoviruses is their high host specificity, which minimizes the risk to non-target organisms, including pollinators and natural enemies, thereby preserving the ecological balance of agroecosystems.

Another BCA that was registered in 2022 was based on the genus *Pseudomonas*, specifically *P. fluorescens* and *P. chlororaphis*, for the biocontrol of 12 pests (*Bemisia tabaci* biotype B, *Dalbulus maidis*, *Euschistus heros, Diaphorina citri, Aphis gossypii, Dichelops melacanthus*, *Leucoptera coffeella*, *Tetranychus urticae*, *Caliothrips brasiliensis*, and *Frankliniella schultzei*). The mode of action of *Pseudomonas* bacteria in pest biocontrol involves multiple pathogenic mechanisms that enable pest infection and colonization. The success of an infection depends on various virulence factors of *Pseudomonas* spp., such as toxin production, biofilm formation, bacterial motility, and gene regulation. The pathogenic effects of these bacteria on insects include physiological alterations, disruption of feeding behavior and developmental processes, physical deformities, and sepsis, which often results in the death of the infected insects (Teoh et al., 2021).

Figure 4C shows the pests with the highest number of registered products. Eighty-three companies registered microbial insecticides with the MAPA, allowing for the commercialization of these products. Simbiose, Koppert, and Biotrop had the highest number of registrations, with 20, 16, and 16, respectively (Figure 4D).

3.3 Microbial nematicides

The registration of nematicides in Brazil has grown significantly over the past few years (Figure 5A; Supplementary Table S2). The number of registrations remained low between 2007 and 2015, reflecting an initially slow adoption of these products with only two registrations (Trichodermil SC and Nemat). However, an upward trend was observed from 2016 onward, with 96 registrations by December 2024, and an average growth of 10 new registrations per

5 https://www.sparcbio.com.br

year. This increase can be attributed to several interrelated factors that drive farmers to adopt these products. One of the main factors is the devastating effects of plant-parasitic nematodes on Brazilian agricultural areas, causing an estimated loss of US$11.7 billion across various crops. Losses due to nematode infestation reach US$5 billion in soybeans, which is equivalent to losing an entire crop every ten years (Machado, 2022).

Another factor contributing to this market's growth is the widespread presence of plant-parasitic nematodes in agricultural regions. A recent survey by Syngenta, Agroconsult, and the Brazilian Society of Nematology revealed that 90% of 21,661 samples collected across Brazil contained plant-parasitic nematodes (Syngenta, 2022). The most prevalent nematode genera in Brazil are *Meloidogyne*, *Pratylenchus*, *Rotylenchus*, and *Heterodera*. These genera cause significant damage to key crops such as soybeans, corn, cotton, and sugarcane, leading to substantial reductions in agricultural productivity and a negative economic impact.

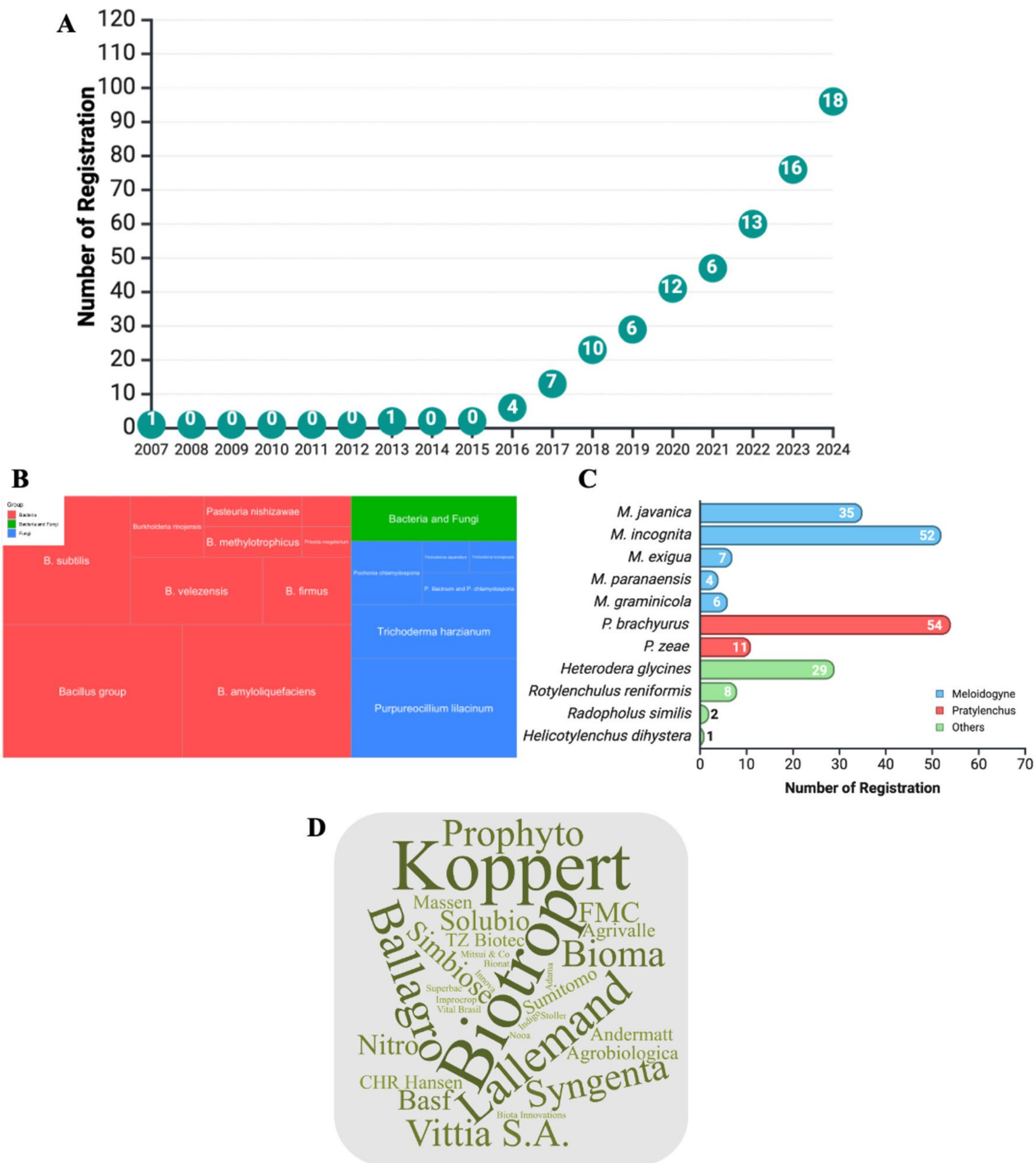

FIGURE 5
Registrations of microbial nematicides in Brazil: Market trends, key pathogens, and microbial species. **(A)** Annual trends in the registration of microbial nematicides in Brazil, demonstrating the increasing adoption of biocontrol agents for nematode management. **(B)** Treemap illustrating the dominant microbial species used for pathogen biocontrol, categorized by organism type (bacteria, fungi, or a combination of both). **(C)** Number of registrations corresponding to the most frequently targeted nematode species, providing insight into the focus on specific disease-causing species. **(D)** Word cloud showcasing the leading companies involved in registering microbial nematicides in Brazil, highlighting the competitive landscape within the industry.

The effectiveness of microbial nematicides in controlling plant-parasitic nematodes and promoting plant growth has been demonstrated in the field, increasing farmers' confidence in, and adoption of, these products (Machado, 2022). Consequently, the market for microbial nematicides will surpass that for chemical nematicides, representing 55, 94, and 100% of the nematicides sold for sugarcane, soybeans, and corn, respectively, in the 2021/2022 season. Projections from Blink Consultancy indicate that this market will continue to grow, with estimated increases of 87, 48, and 86% for the same crops by the 2026/2027 season, attracting the interest of both small and large companies and expanding the range of products available in the market.

In Brazil, the genus *Bacillus* sp. is present in over 60% of commercial microbial nematicide products, either in the consortia of strains from the same or different *Bacillus* species or as single strains of *B. amyloliquefaciens*, *B. velezensis*, *B. subtilis*, and *B. firmus* (Figure 5B). This genus uses various mechanisms to reduce nematode populations, such as (i) regulating nematode behavior by interfering with host recognition, (ii) competing for nutrients, (iii) promoting plant growth, (iv) inducing systemic resistance, and (v) producing metabolites and volatile compounds that inhibit egg hatching, reduce juvenile survival, and directly kill nematodes (Engelbrecht et al., 2018; Aloo et al., 2019; Dimkić et al., 2022; Bhat et al., 2023).

Other bacteria registered for nematode control in Brazil include *Pasteuria nishizawae*, *Pseudomonas oryzihabitans*, and *Burkholderia rinojensis*. The commercial product, Clariva, developed by Syngenta and based on *P. nishizawae*, specifically targets *H. glycines*. In 2023, Indigo registered a new product based on *P. oryzihabitans*, the efficacy of which is associated with producing antimicrobial compounds and cell wall-degrading enzymes, such as chitinases, which directly affect nematode survival and development. Additionally, metabolites of *Burkholderia rinojensis* strain A396, registered by Rizobacter in Brazil and marketed in the United States under the name Majestene® by Marrone Bio Innovations, are noted for their broad-spectrum nematicidal action (Arthurs and Dara, 2019).

Among the fungi registered as microbial nematicides in Brazil, *Purpureocillium lilacinum*, *Trichoderma harzianum*, and *Pochonia chlamydosporia* are notable. These organisms control nematodes through three main mechanisms: direct parasitism, antibiosis, and the induction of plant resistance. *P. lilacinum* and *P. chlamydosporia* primarily target and parasitize nematode eggs. Their hyphae penetrate the egg structure and release enzymes, such as chitinases and proteases, which degrade the protective shell and prevent hatching and juvenile development. Additionally, these fungi produce secondary metabolites with nematicidal properties such as aurovertins and phomalactone, which can kill or inhibit nematode movement (Li et al., 2015; Soares et al., 2023; Ayaz et al., 2024). *T. harzianum* combines both direct and indirect actions for nematode control. In addition to acting as an antagonist by degrading nematode cuticles through lytic enzymes, this fungus also stimulates the natural defenses of plants. By activating the jasmonic acid and salicylic acid signaling pathways, *T. harzianum* induces systemic resistance in plants, making them less susceptible to nematode attacks. Another important mechanism is the production of volatile compounds and secondary metabolites that have direct nematicidal properties or alter the conditions of the rhizosphere, making the environment less favorable for nematode survival (Yao et al., 2023).

The primary targets for microbial nematicide registration in Brazil include *Pratylenchus brachyurus*, *Meloidogyne incognita*, *M. javanica*, and *Heterodera glycines*, with 54, 52, 35, and 29 registered products, respectively (Figure 5C). *Pratylenchus*, particularly *P. brachyurus*, is a migratory endoparasitic nematode that parasitizes many plant species, including soybeans, corn, cotton, rice, and beans. By feeding on the cellular contents of the roots, this nematode causes lesions that lead to necrosis and impaired plant development, resulting in significant yield losses. *P. brachyurus* has emerged as a critical phytosanitary threat in Brazil, especially to soybean production, and can cause crop losses of up to 30%. This impact is amplified in the Cerrado regions, where consecutive planting of soybeans and corn favors the spread of the pathogen. Additionally, *P. brachyurus*' ability to survive for long periods without a host, coupled with its asexual reproduction, complicates its management (Nomura et al., 2024).

Currently, 31 companies have registered microbial nematicides with the MAPA, authorizing the commercialization of these products in Brazil. Biotrop and Koppert led the market with 14 and 9 registrations, respectively (Figure 5D). However, there are duplications in company registrations that artificially increase the total number of registered products. For example, the *B. subtilis* strain CNPSo 3,602 has six different registrations, and *T. harzianum* strain 1,306 has four registrations. This practice of multiple registrations for the same strain contributes to the inflation of registration numbers in the country, thus masking the actual diversity of products available in the market.

3.4 Microbial fungicides

Following the global trend of increasing use of biological products, the microbial fungicide market has experienced accelerated growth in recent years. Between 2018 and 2024, 107 new products were registered, representing approximately 90% of current microbial fungicides (Figure 6A; Supplementary Table S3). The first microbial fungicide registered in Brazil was *T. harzianum* strain ESALQ 1306 in 2007. Subsequently, *T. asperellum* strains URM-5911 and T211, along with the first bacterial strains, *B. pumilus* QST 2808 and *B. subtilis* QST 713, were registered in 2011. These two microorganisms dominate the fungicide market in Brazil (Figure 6B).

Fungi of the genus *Trichoderma* are widely recognized for their fungicidal properties and roles in the biological control of plant diseases. In Brazil, approximately 57% of the registered biofungicides contain fungi of this genus, with the primary biocontrol targets being: (i) *Fusarium oxysporum* f. sp. *phaseoli*, (ii) *Colletotrichum lindemuthianum*, (iii) *Rhizoctonia solani*, and (iv) *Sclerotinia sclerotiorum*. Trichoderma uses both direct and indirect mechanisms for biocontrol of phytopathogenic fungi. Directly, *Trichoderma* competes with pathogens for space and nutrients, particularly in the rhizosphere, and produces secondary antifungal metabolites such as gliotoxin and 6-pentyl-α-pyrone, which inhibit pathogen growth. Mycoparasitism is another important biocontrol mechanism in which *Trichoderma* secretes lytic enzymes, such as chitinases, glucanases, and proteases, which degrade the cell walls of phytopathogenic fungi, leading to their destruction and death. *Trichoderma* induces systemic resistance in plants by activating defense pathways such as jasmonic acid and ethylene. This "priming" effect strengthens the plant's immune response, making it more resistant to pathogen attacks by

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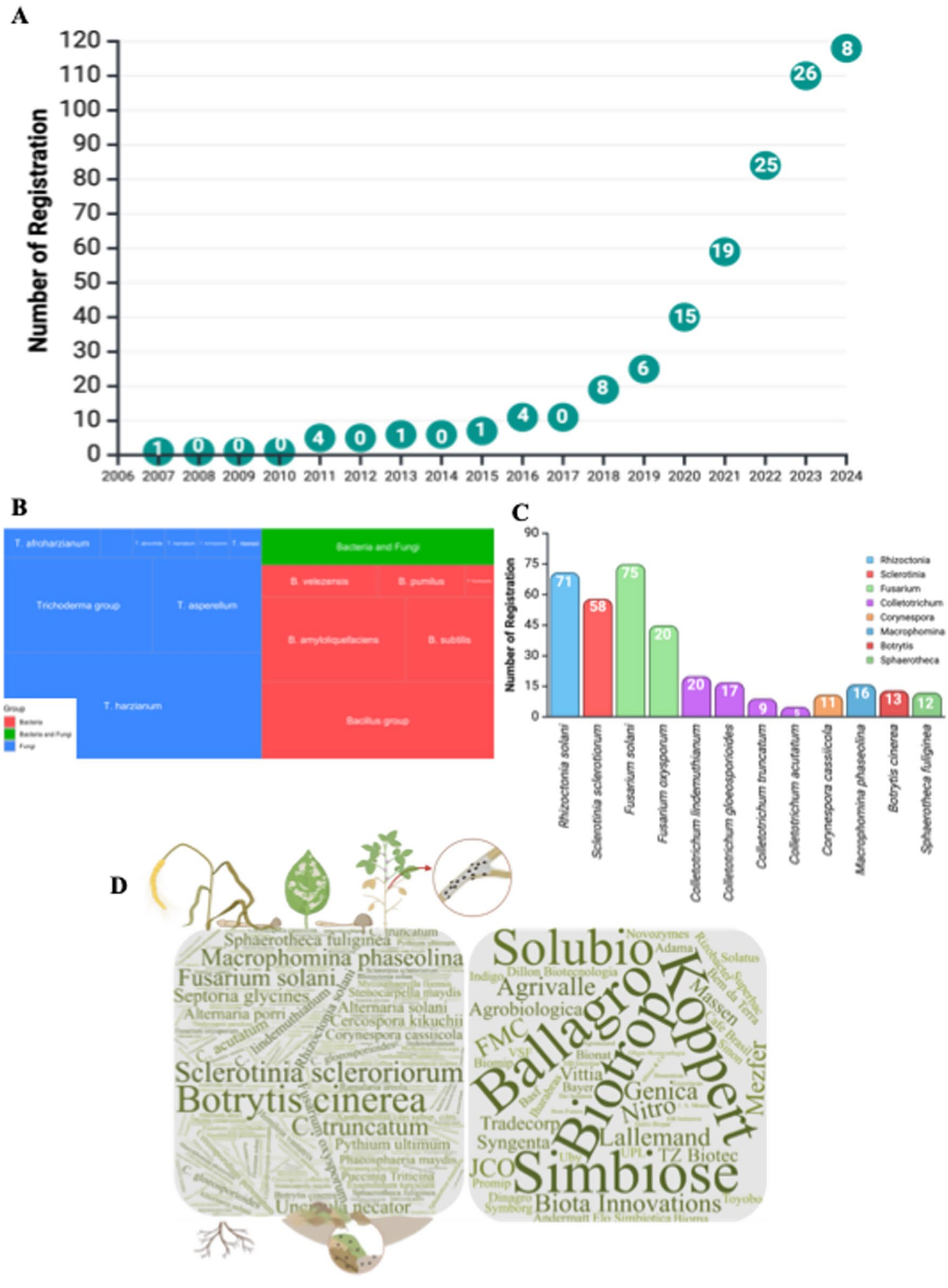

FIGURE 6
Registrations of microbial fungicides in Brazil: Market trends, key pathogens, and microbial species. **(A)** Annual trends in the registration of microbial fungicides in Brazil, demonstrating the increasing adoption of biocontrol agents for disease management. **(B)** Treemap illustrating the dominant microbial species used for pathogen biocontrol, categorized by organism type (bacteria, fungi, or a combination of both). **(C)** Number of registrations corresponding to the most frequently targeted pathogens, providing insight into the focus on specific disease-causing species. **(D)** Word cloud illustrates the pests with the highest number of microbiological fungicide registrations and Brazil's leading companies involved in their registration, highlighting the industry scenario.

increasing the production of pathogenesis-related proteins and other defense enzymes (Asad, 2022; Dutta et al., 2022; Rodrigues et al., 2023). In Brazil, *T. harzianum* and *T. asperellum* species have the highest number of registered products, followed by *T. viride*, *T. hamatum*, *T. afroharzianum*, and *T. atroviride*.

Trichoderma, like entomopathogenic fungi, is traditionally grown on solid substrates, a method known for its simplicity and low cost that facilitates the entry of new companies into the market. This method also promotes the production of high-quality spores with longer shelf lives. However, this method has significant limitations in terms of scalability and automation. In contrast, liquid fermentation offers clear advantages for large-scale production, allowing precise control of variables such as pH, temperature, and aeration and optimizing both fungal growth and the production of secondary metabolites. This results in a more uniform and efficient biomass production over a shorter period than solid substrate fermentation. However, liquid fermentation is more expensive, and the propagules produced have a reduced shelf life, presenting a commercial challenge (Prakash and Basu, 2020). Recent studies have sought to extend the durability of conidia, mainly by adding osmoprotectants to formulations (Sriram et al., 2011; de Rezende et al., 2020; Martinez et al., 2023). Currently, the most common formulations of *Trichoderma*-based products in Brazil are WP and suspension concentrate (SC), which account for approximately 73% of the products on the market. However, in recent years, new formulations have been developed, such as water-dispersible granules (WG), soluble liquid (SL), oil-dispersible (OD), emulsifiable concentrate (EC), emulsifiable gel (GL), and dry suspension (DS), which expand the application options and enhance the use of these biofungicides.

Bacillus species available in the Brazilian market can be grouped into four types: *B. amyloliquefaciens*, *B. velezensis*, *B. subtilis*, and *B. pumilus*, which are formulated either individually or in combination with one or more strains. This genus produces a wide range of antimicrobial compounds, including cyclic lipopeptides, such as iturins, fengycins, and surfactins, destabilizing fungal cell membranes and leading to cell death. Additionally, *Bacillus* spp. secrete lytic enzymes, such as chitinases and glucanases, which degrade fungal cell walls and inhibit fungal growth. Indirectly, *Bacillus* induces systemic resistance in plants by activating defense pathways and enhancing immune responses against fungal pathogens. Furthermore, *Bacillus* produces volatile organic compounds such as acetone, inhibiting fungal growth and spore germination, causing morphological changes in fungal cells (Penha et al., 2020; Hirozawa et al., 2023; Serrão et al., 2024). BCAs containing *Bacillus* strains that are registered in Brazil have demonstrated efficacy against 58 fungal pathogens, including soil-borne pathogens, such as *R. solani*, *S. sclerotiorum*, *M. phaseolina*, and *F. solani*. Additionally, these BCAs are effective against foliar fungi and late-season disease-causing pathogens such as *Botrytis cinerea*, *Phakopsora pachyrhizi*, *Sphaerotheca fuliginea*, *Colletotrichum gloeosporioides*, *C. lindemuthianum*, *Corynespora cassiicola*, and *Cercospora kikuchii*.

Historically, the primary targets for BCA registration in Brazil have been soil-borne diseases (Figure 6C). However, these products have grown in adoption for the integrated management of foliar diseases, acting as protective and preventive agents. Some companies now recommend applying biological products during stages V0–V4 for late-season diseases in soybean cultivation to ensure early protection and increase management effectiveness. When combined with chemical fungicides, biological agents offer comprehensive and complementary multisite actions, reducing the risk of pathogen resistance. This comprehensive approach instills confidence in the audience regarding the effectiveness of products. In addition, these products have prolonged residual effects, contributing to more sustainable and efficient long-term management strategies.

Figure 6D shows the central diseases with the most registered products and the companies with the most registrations. According to the MAPA, 58 companies registered microbial fungicides, with Biotrop leading with 13 registrations, followed by Simbiose, Koppert, and Ballagro with 8, 6, and 6 registrations, respectively.

4 Future perspectives of biopesticides in Brazil

The outlook for Brazil's biopesticide market indicates continued growth. The sector expanded by 312% from 2019 to 2024 (5 years), reaching a revenue of US$737 million. Over the next 5 years (from 2024 to 2029), Brazil is expected to lead the global bioinputs market, with the national market projected to reach US$2.71 billion by 2029 and US$3.11 billion by 2030 (Figure 1). This growth is driven by the increasing integration of biotechnological innovations, increasing adoption by farmers, and the need to reduce dependence on chemical pesticides.

Advancing Brazilian legislation and government incentives are fundamental for expanding the biopesticide market. A milestone in this effort was the establishment of the National Bioinput Program in 2020, aiming to accelerate the development and adoption of microbial biopesticides. The key objectives include proposing regulatory frameworks, fostering scientific and technological innovation, expanding access to credit, promoting biofactories, and providing technical training.

In the current landscape of technological developments, several research fronts are being explored to boost the use of biopesticides in agriculture (Figure 7). Among these, we highlight the following.

4.1 Bioprospecting for new biocontrol agents

The exploration of new strains and microbial species is an important strategy in the development of microbial biopesticides. The discovery of unknown microorganisms, or even those that are already known but underexplored, could reveal BCAs with new applications, thereby increasing the currently available biological control options for pests and diseases (Poveda, 2021). Additionally, advancements in genomic tools, such as metagenomics and next-generation sequencing, have accelerated the identification of bioactive genes and metabolites that can be harnessed for use as biological insecticides, thereby expanding alternatives for pest and disease management (Massart et al., 2015; Zhang et al., 2021). Integrating these tools with the growing understanding of symbiotic and antagonistic interactions in agricultural ecosystems makes it possible to develop more effective biopesticides capable of working synergistically with natural soil microbial communities (Pasin et al., 2021).

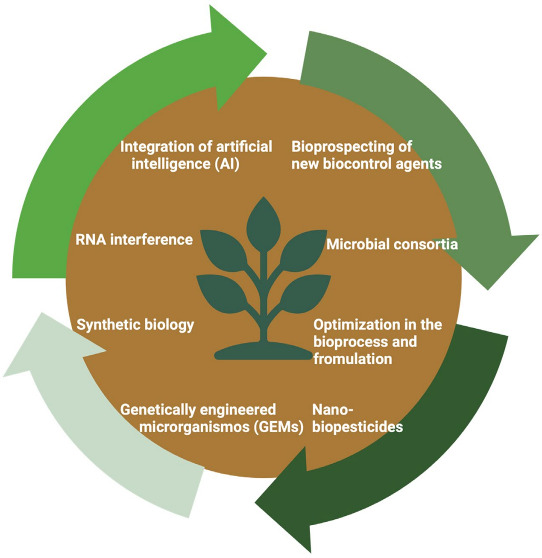

FIGURE 7
Technologies to drive the development of biopesticides for agriculture.

4.2 Microbial consortia

The development of microbial consortia represents a significant advancement in agricultural biopesticides by providing more effective and resilient pest and disease management strategies. Combining multiple microbial species with complementary functions generates synergistic interactions that enhance biocontrol efficacy and improve plant resilience to biotic and abiotic stresses, including pathogen attack and adverse environmental conditions (Nunes et al., 2024). Microbial consortia optimize key physiological processes such as plant growth promotion and nutrient uptake, while reinforcing plant immunity against pathogens, often outperforming biopesticides based on single strains. In addition to their direct benefits to plant health, these microbial formulations enrich soil biodiversity and foster more stable and sustainable agricultural ecosystems. However, the widespread adoption of microbial consortia requires overcoming critical challenges, such as ensuring species compatibility, maintaining product stability, and mitigating competition with native soil microbiomes (Vishwakarma et al., 2020; Nunes et al., 2024).

4.3 Optimization in bioprocessing and formulations

One of bioprocessing's main focuses is optimizing microbial metabolite production for biocontrol through adjustments to parameters such as nutrient availability and fermentation conditions. These adjustments can significantly improve the yield and stability of the metabolites. Another important aspect is the optimization of liquid fermentation for entomopathogenic fungi, such as *B. bassiana* and *M. anisopliae*, to increase the production of blastospores. However, a principal challenge remains in extending the shelf life of these blastospores, which degrade quickly, thereby limiting their prolonged field application (Mascarin et al., 2024). Microencapsulation has emerged as a promising strategy for protecting microorganisms and their metabolites in soil. This technique encapsulates biological agents in a protective matrix, shielding them from competition with native microbiota and adverse environmental conditions, while allowing for more controlled and gradual release. This enhances their stability, competitiveness, and efficacy in pest and disease control. For foliar applications, the challenge is to protect microorganisms from environmental conditions, such as humidity, UV radiation, and temperature. Research on formulations incorporating protective and stabilizing agents has shown great potential to ensure that microorganisms and their metabolites remain active under different environmental conditions, maximizing their effectiveness in the field.

4.4 Nano-biopesticides

Nanotechnology is an important area for the development of biopesticides, with the advent of nanobiopesticides providing advances in terms of stability, controlled delivery, and greater efficacy, allowing the use of smaller doses (Ayilara et al., 2023; Sreevidya et al., 2023). These nanoformulations overcome classical limitations, such as rapid degradation, inconsistent performance, and high production costs, which have historically restricted the widespread adoption of microbial pesticides. By encapsulating microbial agents in nanoparticles, these technologies offer protection against environmental degradation, increase the persistence of the agents in the field, and ensure precise release at the site of action. Additionally, the increased surface area of nanoparticles enhances their interaction with pathogens, boosting bioactivity and maximizing efficacy (Sreevidya et al., 2023).

4.5 Genetically modified microorganisms or genetically edited microorganisms

Genetic engineering techniques, such as genomic recombination and gene editing, have been successfully applied to various microorganisms, allowing for precise modification of genes associated with biocontrol activity (Panth et al., 2020; Liu et al., 2022; Puan et al., 2023; Wei et al., 2024). According to Puan et al. (2023), various genetic engineering approaches have been developed to increase the production of antimicrobial metabolites by *Bacillus* spp. Key strategies include modifying promoters and genetic expression systems that enhance the transcription of genes involved in the biosynthesis of antimicrobial peptides (AMPs), such as surfactin, iturin, and fengycin, which are known for their action against agricultural pathogens. Similarly, in *Trichoderma*, genetic engineering promotes the overproduction of lytic enzymes that degrade the cell walls of pathogens and stimulates the synthesis of bioactive compounds with potent antifungal activities (Adnan et al., 2022; Xiao et al., 2023).

Using microbial chassis, such as *Escherichia coli* and *Saccharomyces cerevisiae*, has shown promise for the heterologous production of complex metabolites with high potential as biopesticides. These chasses are robust platforms for large-scale production of agriculturally relevant compounds, opening new frontiers for developing more efficient biological products. However, the use of genetically modified microorganisms (GEMs) raises essential questions regarding their environmental impacts and food safety. Concerns include the potential adverse effects on natural ecosystems, such as unintended interactions with non-target organisms and the spread of modified genes (Shams et al., 2024). In Brazil, the registration and commercialization of microorganisms must undergo risk assessment and obtain prior authorization from the National Technical Biosafety Commission (CTNBio), depending on the assigned risk class. Activities must follow appropriate containment and biosafety protocols, with protection levels ranging from NB-1 to NB-4 according to the level of risk involved. These protocols are designed to minimize the potential escape or accidental release of organisms into the environment.

4.6 Synthetic biology

Synthetic biology can revolutionize the development of microbial biopesticides in agriculture, thereby enabling the design and engineering of microbial systems with enhanced functionality and precision (Sargent et al., 2022; Wang et al., 2022; Sreevidya et al., 2023). Synthetic biology facilitates the simultaneous introduction of multiple traits such as enhanced resistance to pathogens, by enabling the redesign of existing biological systems or the creation of new genetic circuits (Sargent et al., 2022). This capability is crucial for developing next-generation microbial consortia and biopesticides that are more tolerant to environmental stresses and more effective in protecting crops (Ke et al., 2021; Basnet et al., 2022). Moreover, the synthetic biology of biopesticides can accelerate the discovery of new metabolic pathways and bioactive compounds, thereby expanding the arsenal of tools available for biocontrol.

4.7 RNA interference

RNAi technology utilizes double-stranded RNA (dsRNA) molecules to silence specific genes in target organisms, blocking gene expression at the mRNA level and preventing the production of proteins essential for the development of agricultural pests (Guan et al., 2021; Sharma et al., 2023). This mechanism offers an advantage over conventional chemical pesticides by allowing non-target organisms and beneficial species to remain unharmed. The production of dsRNA in microorganisms, such as *E. coli* and *S. cerevisiae*, has made RNAi-based biopesticides scalable and economically viable (Fletcher et al., 2020; Guan et al., 2021). However, field applications face challenges such as the rapid degradation of dsRNA under adverse environmental conditions, especially when exposed to UV radiation and moisture. Moreover, the efficacy of RNAi varies across insect species and is particularly low in lepidopterans, which exhibit limited dsRNA uptake (Sharma et al., 2023). Recent advancements in RNAi technology, such as the encapsulation of dsRNA in nanoparticles and their conjugation with polymers, have improved the stability and uptake of these molecules, thereby increasing the effectiveness of RNAi in the field (Dalakouras et al., 2024). The U. S. EPA approved the first commercial RNAi-based insecticide, called Calantha, which was developed by GreenLight Biosciences, in 2024. The active ingredient ledprona is a dsRNA molecule that interferes with the expression of the *Snf7* gene in the Colorado potato beetle (*Leptinotarsa decemlineata*), a gene essential for the function of the ESCRT-III complex, which is responsible for cellular protein degradation. Disruption of this gene leads to cellular dysfunction and eventually the death of the insect, offering a specific and effective control for this pest.

4.8 Integration of artificial intelligence

Artificial intelligence (AI) offers an innovative and promising approach for developing new microbial pesticides. AI-based tools, such as machine learning, deep learning, predictive modeling, and genetic algorithms, can significantly accelerate bioprospecting by analyzing large volumes of genomic data and identifying microbial species, strains, and genes with high biocontrol potential. This capability enables predicting functional interactions and efficient identification of new bioactive compounds. Additionally, AI can be used to optimize the microbial consortium design and maximize the synergistic interactions between different species or strains, resulting in more effective consortia for biocontrol. AI-driven synthetic biology tools facilitate the creation of genetically modified microorganisms with stability, resilience, and specificity for the control of pests and pathogens (Li et al., 2021; Holzinger et al., 2023).

5 Concluding remarks

The biopesticide market in Brazil, favored due to the country's rich biodiversity and the increasing global demand for reducing agrochemicals in food production, has grown rapidly and will continue to grow. This, supported by innovations and the discovery of new green-control products, makes the country the global leader in the development and adoption of this technology. The replacement of traditional chemical pesticides with bioinputs has transformed agriculture in the country. In a continuous search to increase Brazilian agricultural sustainability, adopting IPM and IDM must be incentivized and expanded nationwide. IPM and IDM promote a more rational use of pesticides and, therefore, a more balanced and favorable agroecosystem for biocontrol. IPM and IDM adoption increase the chances of biocontrol to survive and persist in agroecosystems, thereby promoting sustainable and profitable agriculture. Continuous investments in public and private research and the training of different actors in different production systems of agriculture will facilitate and consolidate the continuously growing biological control market in the country.

Author contributions

MF: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. SM: Conceptualization, Formal analysis, Investigation, Writing – original draft. GA: Writing – review & editing. AF: Writing – review & editing. MV: Writing – review & editing. JM: Writing – review & editing. EF: Writing – review & editing. MM: Conceptualization, Investigation, Methodology, Writing – original draft. AS: Conceptualization, Investigation, Methodology, Writing – review & editing. RR: Writing – review & editing. LG: Conceptualization, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing.

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Conflict of interest

EF is the biologicals manager at BRANDT BRASIL; SM, MM, and AS are researchers at BIOINPUT; and RR is the CEO of BIOINPUT.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2025.1574269/full#supplementary-material

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Artigo III

Prospecção, caracterização e coinoculação de bactérias solubilizadoras de fosfato e o fungo *Rhizophagus clarus* em casa de vegetação na cultura do algodão

Matheus Felipe de Lima Andreata, Galdino Andrade*

* Laboratório de Ecologia Microbiana, Departamento de Microbiologia, Universidade Estadual de Londrina, Londrina, CEP 86057-970, Brasil.

Resumo

A agricultura moderna enfrenta desafios significativos em relação ao uso excessivo de fertilizantes químicos, que, embora tenham impulsionado a produtividade, resultam em problemas ambientais, como degradação do solo e contaminação de recursos hídricos. Os fertilizantes fosfatados, em particular, apresentam baixa eficiência de utilização pelas plantas, com grande parte do fósforo (P) permanecendo no solo em formas insolúveis. Nesse contexto, a busca por alternativas biológicas, como microrganismos promotores de crescimento vegetal tem se intensificado. Este estudo teve como objetivo prospectar isolados bacterianos capazes de solubilizar fosfato, provenientes da rizosfera de milho e de esporos micorrízicos, e avaliar sua capacidade de promover o crescimento vegetal. Os isolados foram caracterizados *in vitro* quanto à produção de ácido indolacético (AIA), solubilização de fosfato, produção de sideróforos, atividade enzimática e capacidade de colonização das raízes de tabaco. Dois isolados foram selecionados e identificados como *Micrococcus sp.* cepa BE1 e *Lysinibacillus sp.* cepa SF1, e avaliados quanto à produção de ácidos orgânicos em fermentação líquida e à promoção de crescimento na cultura do algodão, tanto isolados quanto em associação com o fungo micorrízico arbuscular (FMA) *Rhizophagus clarus*. *In vitro*, os resultados indicaram um potencial das cepas selecionadas como promotoras de crescimento vegetal a partir de diferentes mecanismos, como a solubilização de fosfato, produção de AIA e uma produção de ácidos orgânicos significativa. Em casa de vegetação na cultura do algodão, apesar de SF1 aumentar os níveis de P (63%) e BE1 os níveis de N (20%) de folha, não constatamos aumentos significativos de biomassa. Quando coinoculados com *R. clarus*, as cepas não demonstraram potencial sinérgico.

Palavras-chave: Ácido málico; ácido glucônico; PGPR; PGPF.

1. INTRODUÇÃO

Com vastas extensões territoriais e um clima favorável para uma grande variedade de cultivos, o Brasil desempenha um papel crucial na agricultura global, destacando-se como um dos principais produtores e exportadores de *commodities* agrícolas do mundo. Além disso, o setor agrícola brasileiro desempenha um papel central na economia nacional, gerando empregos, impulsionando o crescimento econômico e promovendo o desenvolvimento rural em diversas regiões do país. No ano de 2023, o agronegócio foi responsável por cerca de 23,8% do Produto Interno Bruto (PIB) brasileiro cerca de 2,58 trilhões de reais, sendo 1,86 trilhão no ramo agrícola e 721 bilhões no ramo pecuário (CEPEA, 2024).

A utilização de insumos químicos foi essencial para que o Brasil se tornasse uma potência agrícola e ainda é uma importante ferramenta para manutenção de produtividade, seja na disponibilização de nutrientes a partir da adubação química ou no controle de pragas por pesticidas. Porém, é conhecido o impacto negativo desses insumos sobre a qualidade do solo, água e a saúde humana, neste panorama, têm crescido as exigências por uma agricultura mais sustentável (Yadav and Devi, 2017; Baweja et al., 2020).

Quando falamos de fertilizantes químicos, o Brasil está entre os maiores consumidores desses produtos, cerca de 8% mercado global de fertilizantes, gerando um gasto anual de 25 bilhões de dólares, o que destaca uma alta dependência externa que expõe o principal setor econômico do Brasil a flutuações no mercado internacional de fertilizantes (Brasil, 2022). Os fertilizantes fosfatados apresentam as menores taxas de eficiência de aplicação, apenas 15% a 50% do fósforo aplicado é absorvido pelas plantas. Por esse motivo, os solos brasileiros contêm concentrações elevadas de fósforo em formas insolúveis, consequência de décadas de fertilização em solos ricos em ferro (Fe) e alumínio (Al), culminando na rápida imobilização desse nutriente. Estima-se que, até 2018, o Brasil tenha acumulado 33,4 teragramas (Tg) de fósforo em seus solos. Esse reservatório acumulado de fósforo possui um valor econômico estimado de 22 bilhões de dólares (Pavinato et al., 2020).

Em 2022, o Governo Federal Brasileiro lançou o Plano Nacional de Fertilizantes (PNF), com o objetivo de reduzir a importação desses insumos de 85% para 45% até 2050. O desenvolvimento de insumos biológicos mais eficientes é uma das estratégias delineadas no plano (Brasil, 2022).

Na última década, a utilização de produtos biológicos na agricultura, com destaque para os microrganismos, tem se tornado cada vez mais comum, sendo um recurso essencial para cultivos mais sustentáveis. A expectativa é que o mercado de biodefensivos que em 2021 girava em torno de 1,3 bilhão de reais, atinja um valor de 17 bilhões em 2030, esse avanço tem sido impulsionado por políticas públicas que incentivam a pesquisa e desenvolvimento, registro de novos produtos biológicos, além de uma grande adesão do mercado consumidor (Borsari e Vieira, 2022).

A inoculação de fungos micorrízicos arbusculares (FMA) e bactérias solubilizadoras de fosfato (BSF) é uma alternativa importante para aumentar a eficiência da absorção de fósforo, reduzindo a necessidade de aplicação e utilizando o fósforo acumulado no solo (Begum et al., 2019; Bhupenchandra et al., 2024; Li et al., 2023). No entanto, a aplicação desses microrganismos ainda não está amplamente disseminada no Brasil. Por exemplo, os produtos de FMA disponíveis são importados e não utilizam cepas nativas do país, destacando uma lacuna no investimento em tecnologias para pesquisa de cepas e no desenvolvimento de sistemas de produção desses microrganismos. Quanto as bactérias solubilizadoras de fosfato, apesar de apresentarem maior disponibilidade de produtos no mercado, ainda tem baixa adesão do mercado consumidor.

É importante a busca por novas tecnologias que aumentem a eficiência desses produtos no campo. A coinoculação de FMA e BSF é uma alternativa, visto que os FMA, além de promoverem diversos benefícios diretos a planta, também estimulam a microbiota ao redor de suas hifas, a partir da maior formação de agregados e consequentemente maior retenção de nutrientes, e pela liberação de sinalizações químicas através das hifas (Wahid et al., 2016; Nacoon et al., 2020; Mirzaei Heydari et al., 2024; Wang et al., 2024). São necessárias pesquisas que busquem investigar a interação entre cepas de FMA e BSF, visando uma atuação sinérgica a fim de aumentar a eficiência da absorção do fósforo no solo pelas plantas.

Com base nisso, este trabalho teve como objetivo prospectar isolados capazes de solubilizar fosfato, caracterizar a atividade de promoção de crescimento *in vitro* e avaliar a sua capacidade de promover crescimento vegetal associado ao FMA *Rhizophagus clarus* em casa de vegetação na cultura do algodão.

2. MATERIAIS E MÉTODOS

2.1. OBTENÇÃO DE ISOLADOS

Os isolados foram obtidos de duas fontes distintas, esporos do FMA *Rhizophagus clarus* (BE) e a partir de solo rizosférico de plantas de milho (SF). Os esporos do FMA *Rhizophagus clarus* provenientes de vasos-cultura, mantidos em associação com *Brachiaria decumbens*. Em vasos de 1L, foi utilizado como substrato uma mistura de solo e areia na proporção 3:1, e realizada adubação líquida de 10 mL de nitrogênio (N), fósforo (P) e potássio (K) na proporção 20:10:20, a cada 15 dias. As culturas foram mantidas por 90 dias com rega diária. Após esse período, a irrigação foi interrompida por 15 dias para esporulação. Os esporos foram extraídos do solo pelos métodos de peneiramento úmido e gradientes de densidade com solução de sacarose 30% (Saggin Júnior et al., 2011).Após separados, 50 esporos foram manualmente coletados com auxílio de estereoscópio óptico Motic SMZ (40×). Para a desinfecção da superfície, os esporos foram mantidos sob agitação em solução de cloramina T 2% (m/v), estreptomicina 0,2% (m/v) e 0,002% de polisorbato 20 por 20 minutos. Posteriormente, os esporos foram separados por centrifugação 5 minutos a 10.000 rpm (Centrifuga Sorvall RC-5B refrigerada), o sobrenadante foi descartado e os esporos ressuspendidos em água destilada estéril, esse processo foi repetido por 3 vezes (Declerck et al., 2005). Os esporos foram macerados com auxílio de cadinho e pistilo, em condições estéreis. Ao macerado foram adicionados 10 mL de solução salina de NaCl (0,85% m/v) e essa suspensão foi diluída serialmente com plaqueamento das diluições 10^{-1}, 10^{-2} e 10^{-3} em meio ágar nutriente, incubada por 48 horas a 28°C.

Quanto aos isolados SF, amostras de 1 g de solo rizosférico de milho foram pesadas e suspendidas em 99 mL de NaCl (0,85%). Em seguida, as amostras foram serialmente diluídas e plaqueadas em meio Ágar Pikovskaya, para diferenciar microrganismos solubilizadores de fosfato (Gupta et al., 2022), nas diluições 10^{-2} e 10^{-3}. Os isolados BE e SF foram estocadas em meio ágar nutriente com 40% v/v de glicerol a -20 °C.

2.2. SCRENNING *IN VITRO*

2.2.1. Solubilização de fosfato

A capacidade de solubilizar fosfato foi avaliada de forma qualitativa a partir da inoculação de 20 µL de suspensões bacterianas (A 10^8 UFC/mL, 0,8 na escala de

McFarland) em meio Ágar Pikovskaya, em pontos centrais da placa. Após 72 h de incubação a 28°C, a capacidade solubilização de fosfato foi avaliado pela presença ou ausência de um halo transparente ao redor da colônia bacteriana e determinado como positiva (+) ou negativa (-), respectivamente (Gupta et al., 2022).

2.2.2. Produção de AIA (Ácido Indol-3-Acético)

Para quantificação da produção de AIA, tubos contendo 10 mL de meio TSB 10% (m/v) suplementado com L-triptofano (1 g/L) foram inoculados com 20 µL de suspensão bacteriana na densidade de 10^8 UFC/mL (0,8 na escala de McFarland), em seguida, incubados em agitador orbital por 48 h sob 170 rpm e 28°C. As culturas foram centrifugadas a 12.000 rpm durante 10 minutos e alíquotas de 100 µL do sobrenadante foram transferidas para uma placa de 96 poços, onde foram adicionados 100 µL de reagente de Salkowski. Após 30 minutos reagindo, na ausência de luz, foi realizada leitura dos poços em leitor de microplacas em 530 nm. Os valores médios obtidos foram contrastados com a curva de calibração, obtida através da utilização de Ácido indol-3-acético (98%), nas concentrações 0, 5, 10, 20, 60 e 90 µg/mL (Sarwar e Kremer, 1995).

2.2.3. Produção de Exopolissacarídeos (EPS)

A produção de EPS foi avaliada a partir da adição de colônias bacterianas (48 h de incubação sob 28 °C) a 2 mL de etanol (99,5%). A formação de precipitado indica a presença de EPS (+), enquanto a formação de turbidez indica o resultado negativo para a presença de EPS (-) (Paulo et al., 2012).

2.2.4. Atividade enzimática

As cepas foram testadas quanto a capacidade de quebrar amido, celulose e proteínas. Para avaliação as bactérias foram inoculadas em meios diferenciais e incubadas a 28°C por 72 horas. Para a quebra de amido, foi utilizado o meio completo com adição de 2% de amido, após incubação a superfície foi coberta com lugol (0,1 M) por 15 minutos (Pontecorvo et al., 1953). Os meios celulolítico e proteolítico utilizados foram desenvolvidos por Hankin e Anagnostakis (1977) e Martley, Jayashankar e Lawrence (1970) respectivamente. Para revelação do meio celulolítico, a superfície de cultivos foi coberta com NaCl 1 M por 5 minutos. Em seguida, a solução foi removida e adicionada solução de vermelho congo 0,1% (m/v) durante 30 minutos. Para o meio proteolítico não foi necessária adição de soluções para revelação. Para as três avaliações, a formação de um halo transparente ao redor da colônia indica resultado de lise (+), a não formação indica a incapacidade do isolado em quebrar o componente alvo (-).

2.2.5. Colonização de Raíz

A capacidade das cepas bacterianas colonizarem raízes foi avaliada de forma qualitativa, a partir do crescimento das bactérias em contato com raízes de tabaco. Previamente, 50 µL de suspensão bacteriana (0,8 na escala McFarland, diluído serialmente até 10^5 UFC/mL), foi espalhada com auxílio de alça de drigalski em meio ágar-água, posteriormente, foi adicionado um fragmento de raiz de tabaco transformada pela inserção do plasmídeo *Ti* de *Agrobacterium rhizogenes*. As placas foram incubadas durante 7 dias a 25°C. A presença de crescimento bacteriano ao redor da raiz sem supressão do crescimento vegetal foi considerada um indicativo do potencial de colonização da raiz. Essa metodologia foi adaptada de Nihorimbere et al. (2012) e Verma, Chen e White (2022).

2.2.6. Produção de Ácidos Orgânicos Totais

As cepas foram incubadas por 10 dias a 28°C e 120 rpm, em meio NBRIP líquido contendo 5g/L de fosfato tricálcico como fonte de fósforo, seguindo o método descrito por Nahas et al. (1994). A determinação de ácidos orgânicos dos fermentados centrifugados e filtrados foram injetados em sistema cromatográfico Shimadzu modelo LC 20 A (Kyoto, japão) constituído por Bomba de alta pressão LC- 20AT, injetor automático SIL-20AC HT, detector de índice de Refração RID-10A, detector de arranjo de fotodiodos SPD-M20A, forno de coluna CTO-20A e módulo de controle CBM-20A. Para as análises utilizou-se coluna cromatográfica Phenomenex 5µ C18 MG 250 x 4,6mm. A fase móvel consistiu em solução tampão fosfato de sódio 25mM, com pH ajustado para 2,4 na vazão de 1,0 mL min^{-1}, a temperatura da coluna foi mantida a 40°C e o volume de injeção em 20µL. A detecção foi realizada no detector de Arranjo de Fotodiodos (SPD-M20A), programado em comprimento de onda fixo de 205nm e no modo de varredura de 200 a 400nm. A aquisição e processamento dos dados foram realizados com o auxílio do Software Shimadzu LCsolution (Kyoto, Japão) (Oliveira-Paiva, de et al., 2024; Yan et al., 2013).

2.3. IDENTIFICAÇÃO DAS CEPAS

2.3.1. Extração de DNA

Aproximadamente 150 mg de micélio e colônias foram utilizados para extração de DNA utilizando o kit Extracta® Kit DNA e RNA de Patógenos MDx (Loccus) e o extrator e purificador de DNA e RNA Extracta® 32 (Loccus) conforme as recomendações do fabricante. O DNA obtido foi quantificado e avaliado quanto à pureza em NanoDrop® 2000 (Thermo Fisher).

2.3.2. PCR Convencional

A amplificação da região das regiões V3-V4 do RNA ribossomal 16S foi realizada em termociclador Veriti (Thermo Fisher), em reação contendo 20 ng de DNA genômico purificado. Os produtos foram analisados por eletroforese em agarose 1% (m/v) tampão Tris-Acetato-EDTA. As reações foram purificadas enzimaticamente com ExoI/SAP (Thermo Fisher), conforme recomendações do fabricante.

2.3.3. Sequenciamento Sanger

Os produtos de PCR foram marcados com *BigDye* v3.1 (Thermo) conforme orientações do fabricante, em reações contendo 50 ng de DNA. Essas amostras foram sequenciadas pela plataforma *Genetic Analyser* 3500xL (Thermo), utilizando capilares de 50 cm com polímero Pop7 (Thermo Fisher), conforme orientações do fabricante. Os eletroforetogramas gerados foram convertidos em sequência de bases com o software *Sequencing Analysis* 5.4 (Thermo Fisher).

2.3.4. Análise Bioinformática

As sequências obtidas pela conversão dos eletroforetogramas foram trimadas por qualidade utilizando Phred > 25, e a montagem foi realizada utilizando o software CAP3 *sequence assembly program*. O contig gerado foi comparado com banco de dados do NCBI utilizando o software *BLASTn*. Para construção das árvores filogenéticas, foi realizado o alinhamento das sequências obtidas com as sequências disponíveis no *GenBank*, e os níveis de similaridade de sequência foram calculados usando *Clustal Omega*. Edições do alinhamento foram realizadas utilizando o software *Aliview* e as árvores foram construídas pelo método verossimilhança usando o software *IQtree*. A topologia da árvore filogenética foi avaliada usando o método *bootstrap* com 1000 réplicas sendo as árvores desenhadas utilizando a plataforma *iTOL*.

2.4. EXPERIMENTO EM CASA DE VEGETAÇÃO

A atividade de promoção de crescimento vegetal pelas bactérias selecionadas foi investigada por meio de experimento em casa de vegetação em plantas de algodão (*Gossypium hirsutum* L.), cultura que comumente apresenta uma boa resposta a inoculação micorrízica. Foram avaliados 6 tratamentos com 5 repetições, descritos na tabela abaixo (Tabela 1.)

Tabela 1. Descrição dos tratamentos avaliados em experimento em casa de vegetação na cultura do algodão.

Tratamento	Composição	Concentração (50g de turfa)
Controle	Não inoculado	-
Mico	*Rhizophagus clarus*	10000 esporos
SF	*Lysinibacillus* sp. SF1	10^7 UFC/g
SFM	*Lysinibacillus* sp. SF1 + *R. clarus*	10^7 UFC/g + 10000 esporos
BE	*Micrococcus* sp. BE1	10^7 UFC/g
BEM	*Micrococcus* sp. BE1+ *R. clarus*	10^7 UFC/g +10000 esporos

2.4.1. Características do Solo e Cultivo

As plantas de algodão (FM 954 GLT) foram cultivadas em vasos de 5 Kg contendo solo e areia na proporção 3:1, tindalizados por 1 hora em 3 dias consecutivos, para eliminação dos fungos micorrízicos selvagens, com pH final da solução igual a 5,7. Para reposição da comunidade microbiana foi adicionado 10 mL de uma solução de solo (25g/L) filtrada em papel Whatman®. Foram semeadas três sementes por vaso, sendo desbastadas após a germinação (aproximadamente 72 h), restando apenas uma planta. Os níveis de fósforo (P) no solo extraído por digestão nitroperclórica foi estimado em 3,64 ppm de acordo com Murphy e Riley, (1962). A aplicação de adubos foi realizada após a germinação e a cada 15 dias, com 15 mL de solução de N-P-K (20-10-20) e 1 mL de solução de micronutrientes (H_3BO_3, 0,5 mg/L; $MnSO_4 \cdot H_2O$, 0,5 mg/L; $ZnSO_4 \cdot 7H_2O$, 0,5 mg/L; $CuSO_4 \cdot 5H_2O$, 0,01 mg/L;

$Na_2MoO_4 \cdot 2H_2O$, 0,005 mg/L). As plantas foram mantidas a 27 ± 3 °C e a rega das plantas foi realizada diariamente com 250 mL de água comum.

2.4.2. Cultivo e Inoculação de Microrganismos

Os propágulos de FMA foram obtidos a partir de um cultivo axênico *in vitro* entre *Rhizophagus clarus* e raízes de cenoura (*Daucus carota* L.) em meio mineral mínimo (Bécard and Fortin, 1988) por 60 dias a 25 °C. A inoculação foi realizada em mistura turfosa, tendo 10 g (~200 esporos/g) por vaso, adicionados sob as sementes. As bactérias foram cultivadas em meio TSA por 48 horas a 28 °C. Uma colônia de cada bactéria foi coletada e ressuspendida em NaCl 0,85% (m/v) com ajuste à concentração de 1×10^8 UFC/mL pela escala de McFarland, 1 mL da solução foi misturado em 10 g de substrato turfoso (inoculados com FMA nos tratamentos com associação) e adicionado sob as sementes.

2.4.3. Avaliações dos Tratamentos em Casa de Vegetação

As plantas de algodão foram cultivadas por 45 dias e, no tempo final, foram avaliadas quanto a colonização micorrízica, a partir de coloração do terço médio das raízes, realizada de acordo com Phillips e Hayman (1970). As estruturas fúngicas foram contabilizadas com o auxílio de estereoscópio óptico Motic SMZ (40×) pelo método de grid-line (Giovannetti and Mosse, 1980). Para avaliação da promoção de crescimento, foram determinados: altura e entrenó, matéria seca de raiz e da parte aérea. Para este último, as amostras foram armazenadas em sacos de papel e levados para estufa ventilada a 60 °C até peso constante. Foram também determinados os teores de nitrogênio e fósforo das folhas (Kjeldahl, 1888; Murphy and Riley, 1962).

2.4.4. Análise Estatística

Para a avaliação da normalidade dos dados, foi aplicado o teste de Shapiro-Wilk, considerando um nível de significância de $\alpha = 5\%$. A verificação da homogeneidade das variâncias foi conduzida por meio do teste de Bartlett, também com $\alpha = 5\%$. Posteriormente, realizou-se uma análise de variância (ANOVA) e as médias foram comparadas pelo teste de Tukey, utilizando o software R, estabelecendo como nível de significância $p < 0,05$ para o experimento conduzido em casa de vegetação. Nos casos em que os pressupostos de normalidade e homogeneidade não foram atendidos, as distribuições foram analisadas pelo teste de Kruskal-Wallis, considerando-se $p < 0,05$ como estatisticamente significativo, com as médias sendo comparadas pelo teste de Diferença Mínima Significativa (*Least Significant Difference*, LSD).

3. RESULTADOS

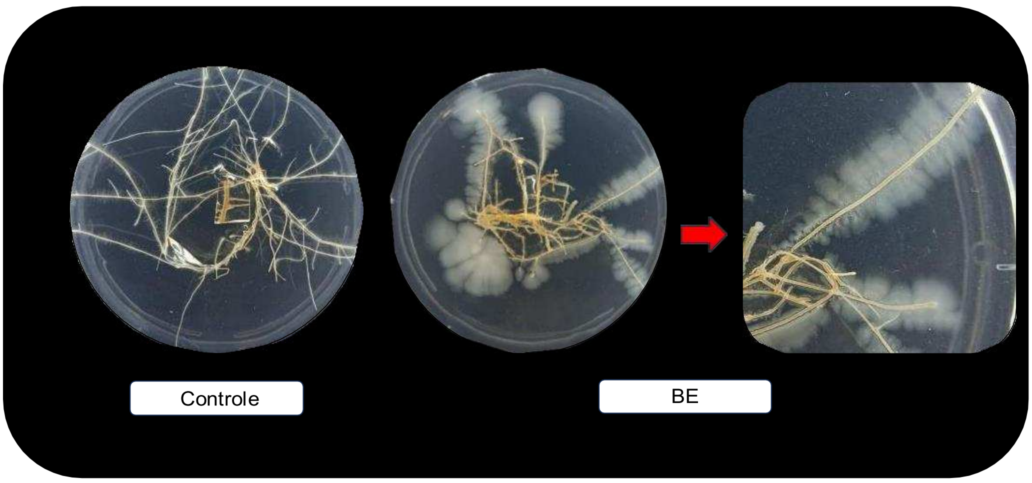
3.1. SCREENING IN VITRO

Além dos 5 isolados SF, selecionados com base na capacidade de solubilizar fosfato, 5 isolados BE apresentaram essa característica, BE1, BE3, BE4, BE5 e BE6. Outra característica importante na promoção de crescimento, a produção de AIA foi detectada com valores entre 5,6 e 15,7 µg/mL, dentre os isolados SF, destaque para SF1 e SF5 (11,7 µg/mL e 12,6 µg/mL, respectivamente). Enquanto para as cepas BE, os maiores valores de produção de AIA foram observados nos isolados BE6 (15,77 µg/mL) e BE5 (14,1 7 µg/mL). Todas os isolados demonstraram capacidade de produzir sideróforos, exceto SF3 e BE3.Também verificamos produção de EPS em 4 isolados (SF1, SF2, SF3 e BE1). Quanto a produção enzimática, nenhum isolado demonstrou capacidade de quebrar celulose, constatamos 5 isolados com capacidade de quebrar proteínas (SF3, SF4, SF5, BE1 e BE4) e 2 isolados com atividade amilolítica (SF4 e BE4). Apenas um isolado demonstrou potencial em colonizar raízes transformadas de tabaco *in vitro* (BE1) (Figura 1). Com base nesses dados e características de crescimento *in vitro*, selecionamos os isolados SF1 e BE1 para identificação em sequenciamento ribossomal 16s, análise da produção de ácidos orgânicos e teste em casa de vegetação (Tabela 2).

Tabela 2. Caracterização dos isolados obtidos quanto ao potencial de promoção de crescimento vegetal *in vitro.*

	SF1	SF2	SF3	SF4	SF5	BE1	BE 2	BE3	BE4	BE5	BE6	BE7
Solubilização de fosfato	+	+	+	+	+	+	-	+	+	+	+	-
Produção de AIA (µg/mL)	11,7	-	-	5,6	12,6	5,0	5,4	10,8	10,5	14,1	15,7	11,55
Produção de sideróforos	+	+	-	+	+	+	+	-	+	+	+	+
Formação de EPS	+	+	+	-	-	+	-	-	-	-	-	-
Atividade proteolítica	-	-	+	+	+	+	-	-	+	-	-	-
Atividade celulolítica	-	-	-	-	-	-	-	-	-	-	-	-
Atividade amilolítica	-	-	-	+	-	-	-	-	+	-	-	-
Colonização de raiz	-	-	-	-	-	+	-	-	-	-	-	-

Figura 1. Avaliação da capacidade do isolados em colonizar raízes de tabaco transformadas pelo plasmídeo *Ti* de *Agrobacterium rhizogenes* em meio ágar-água.



3.2. IDENTIFICAÇÃO DOS ISOLADOS

As sequências 16S rRNA das cepas BE1 e SF1 foram analisadas usando BLASTn no banco de dados GenBank (https://blast.ncbi.nlm.nih.gov). O isolado BE1 exibiu a mais alta similaridade da sequência do gene 16S rRNA com cepas do gênero *Micrococcus*, *Micrococcus aloeverae* (99,79%), *Micococcus yunnanensis* (97,87%), *Micrococcus luteus* (97,62%) e *Micrococcus endophyticus* (97,52%). A árvore filogenética (Figura 2) mostrou que BE1 foi agrupada com cepas de *Micrococcus aloeverae*. Quanto a cepa SF1, exibiu uma alta similaridade com cepas do gênero *Lysinibacillus*, como *Lysinibacillus xylanilyticus* (98,32%), *Lysinibacillus agricola* (97,62%), *Lysinibacillus sphaericus* (96,89%), *Lysinibacillus varians* (96,50%) e *Lysinibacillus tabacifolii* (96,50%). A árvore filogenética mostrou um agrupamento com cepas de *Lysinibacillus xylanilyticus* (Figura 3).

Figura 2. Árvore filogenética da cepa BE1 baseada nas sequências obtidas em sequenciamento Sanger das regiões V3-V4 do RNA ribossomal 16S.

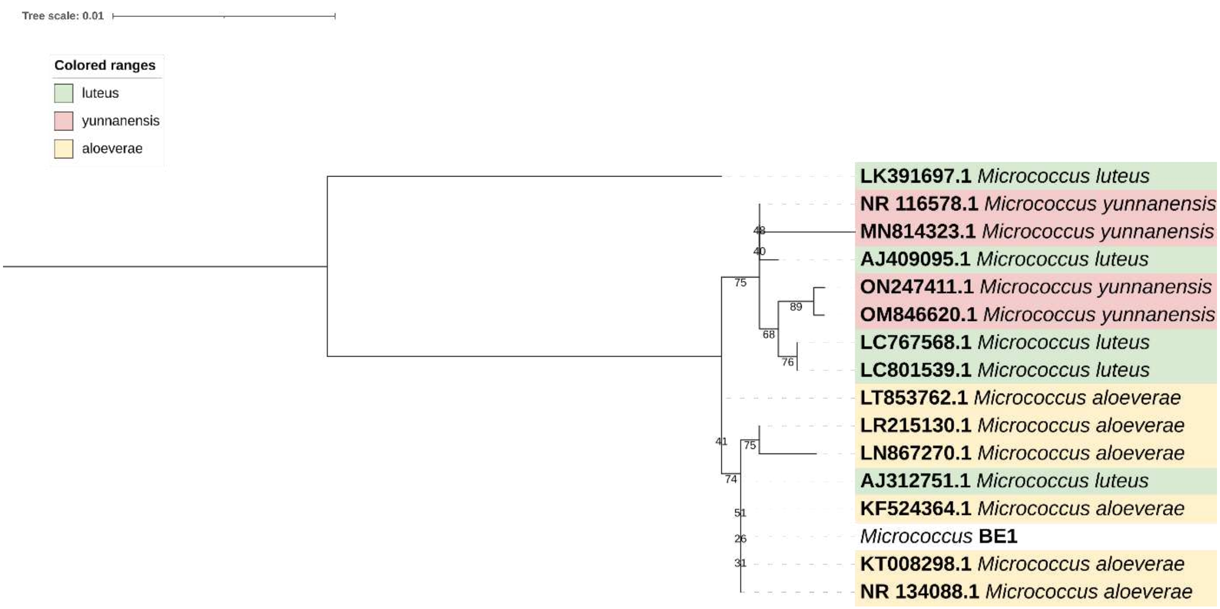
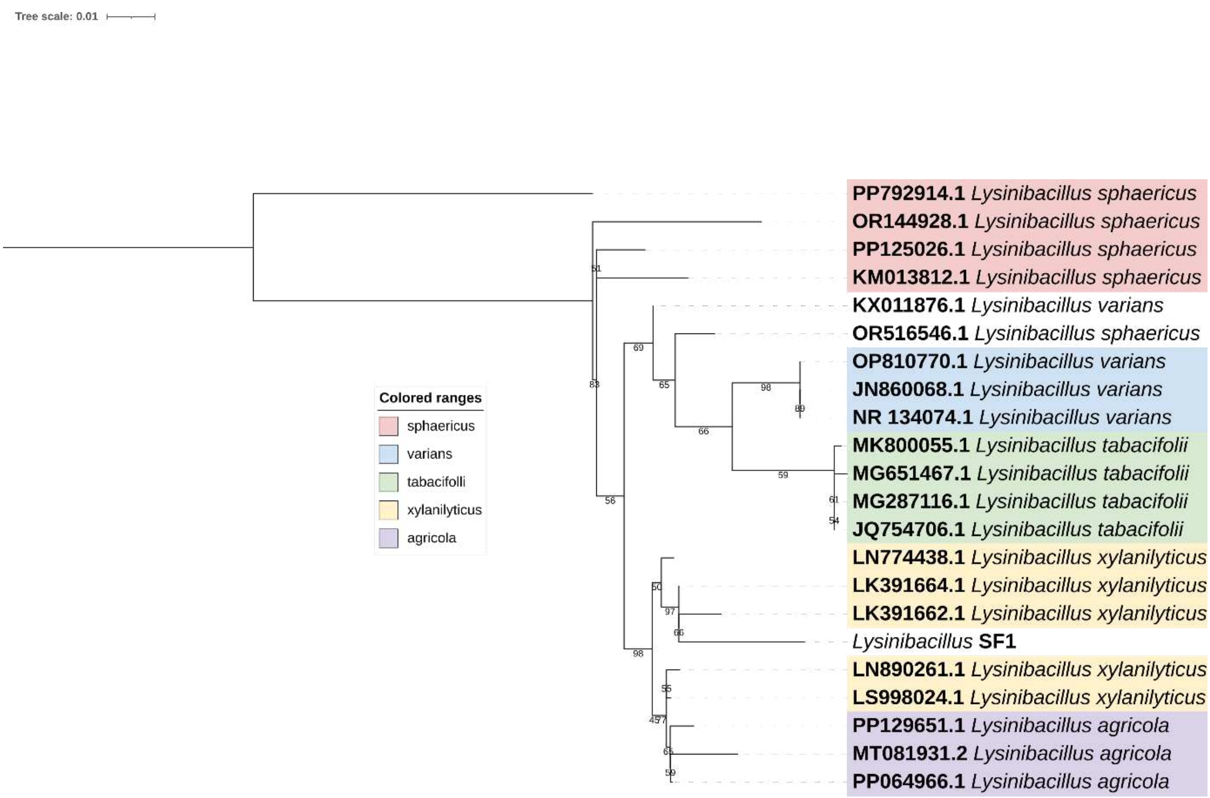


Figura 3 Árvore filogenética da cepa SF1 baseada nas sequências obtidas em sequenciamento Sanger das regiões V3-V4 do RNA ribossomal 16S.



3.3. PRODUÇÃO DE ÁCIDOS ORGÂNICOS

As cepas *Micrococcus* sp BE1 e *Lysinibacillus* sp SF1 apresentaram produção de ácido orgânicos totais, cerca de 158,5 mmol L^{-1} e 182,1 mmol L^{-1}, respectivamente. Foi constatada a produção de ácido glucônico e ácido málico para as duas cepas, sendo na cepa BE1 uma maior produção de ácido glucônico, enquanto para cepa SF1 foi verificado uma maior quantidade de ácido málico (Figura 4 e tabela 3).

Figura 4 – Cromatogramas do controle e dos fermentados das cepas BE1 e SF1 em meio NBRIP com 5g/L de $Ca_3(PO4)_2$ como fonte de P.

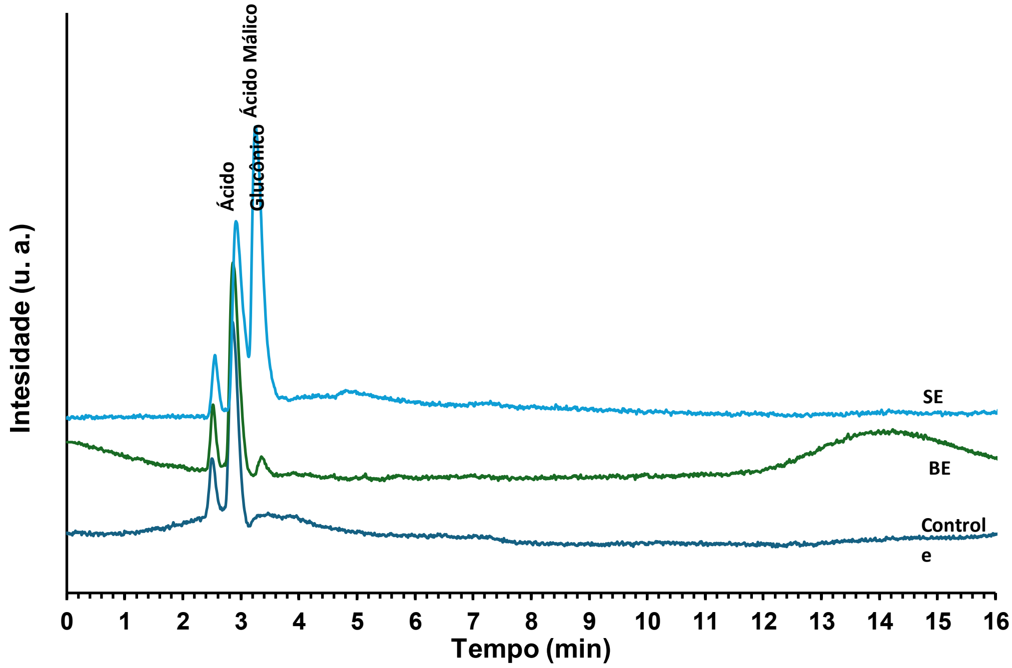


Tabela 3 – Dados das concentrações em 1 mmol L^{-1} dos fermentados das cepas BE1 e SF1 em meio NBRIP com 5g/L de $Ca_3(PO4)_2$ como fonte de P.

Cepa	pH	Ác. Oxálico	Ác. Glucônico	Ác. Málico	Ác. Lático	Ác. Acético	Ác. Cítrico	Ác. Succínico	Total
BE	6,32 ± 0,12	ND	147,7	10,8	ND	ND	ND	ND	158,5
SF	6,16 ± 0,25	ND	48,0	134,1	ND	ND	ND	ND	182,1

ND – Não detectado

3.4. EXPERIMENTO EM CASA DE VEGETAÇÃO

Em casa de vegetação, as cepas selecionadas não demonstraram efeito sobre a colonização micorrízica, com 81,6% de raízes colonizadas no tratamento com *Lysinibacillus* sp. SF1 e 84% com *Micrococcus* sp. BE1, frente a 89,4% no grupo Mico. As plantas coinoculadas com a cepa SF1 e *R. clarus* apresentaram ganhos em média de 24% em altura, 64% em massa seca de parte aérea e 84% em massa seca de raiz em relação ao controle, porém esses valores quando comparados ao tratamento contendo apenas *R. clarus* não apresentaram um aumento significativo, exceto pela altura (17%). As cepas BE1 e SF1, quando inoculadas na ausência de *R. clarus*, não demonstraram potencial de promoção de crescimento na cultura do algodão, no caso da cepa BE1 de *Micrococcus* o efeito sobre o crescimento foi negativo em relação ao número de entrenós e massa seca de raiz (Tabela 4).

Tabela 4. Efeito da inoculação individual e coinoculação de *R. Clarus* e as bactérias *Lysinibacillus* sp. SF1 e *Micrococcus* sp. BE1 sobre a colonização micorrízica, altura, número de entrenós, massa seca de parte aérea e massa seca de raiz. As distribuições foram avaliadas pelo teste de Kruskal-Wallis (p < 0.05) e as médias comparadas pelo teste de LSD (p < 0.05). Média seguidas de letras diferentes apresentam diferença estatística.

	Controle	Mico	SF	SFM	BE	BEM
Colonização (%)	0,00 ± 0,00c	89,40 ± 10,09ab	0,00 ± 0,00c	81,60 ± 5,80a	0,00 ± 0,00c	84,00± 4,00b
Altura (cm)	26,60 ± 3,21bc	27,60 ± 3,36ab	26,60 ± 2,57bc	33,60 ± 0,55a	20,26± 3,40c	31,36± 4,94ab
N° de entrenós	8,80 ± 1,10bc	14,00 ± 1,73a	7,40 ± 1,14cd	10,80 ± 0,45ab	5,20 ± 0,84d	9,20 ± 2,59bc
Massa seca - Parte aéra(g)	2,67 ± 0,73bc	4,35 ± 0,77a	2,72 ± 0,62bc	4,38 ± 0,85a	1,53 ± 0,59c	3,82 ± 1,38ab
Massa seca - Raiz (g)	1,08 ± 0,36bc	1,56 ± 0,22ab	0,80 ± 0,26cd	1,99 ± 0,43a	0,33 ± 0,19d	1,21 ± 0,26bc

Quando comparadas as concentrações de N nas folhas, o tratamento Mico apresentou os níveis mais altos (30,1mg/g) com diferença significativa em relação aos demais tratamentos, incluindo BEM e SFM. Os tratamentos contendo a cepa BE, isolado (24,5 mg/g) ou coinoculado (24,1 mg/g), apresentaram valores significativamente maiores em relação ao controle (20,3 mg/g), não existindo indício de atuação sinérgica quando associado com *R. clarus*. Quanto aos níveis de P nas folhas, a inoculação da cepa SF1 demonstrou potencial em aumentar a disponibilidade de P para planta em cerca de 63% em relação ao grupo controle (Figura 2).

Figura 4. Concentração de fósforo (mg/g) das folhas de algodão, estimado após 45 dias de cultivo em casa de vegetação. Letras diferentes representam diferença significativa entre as médias.

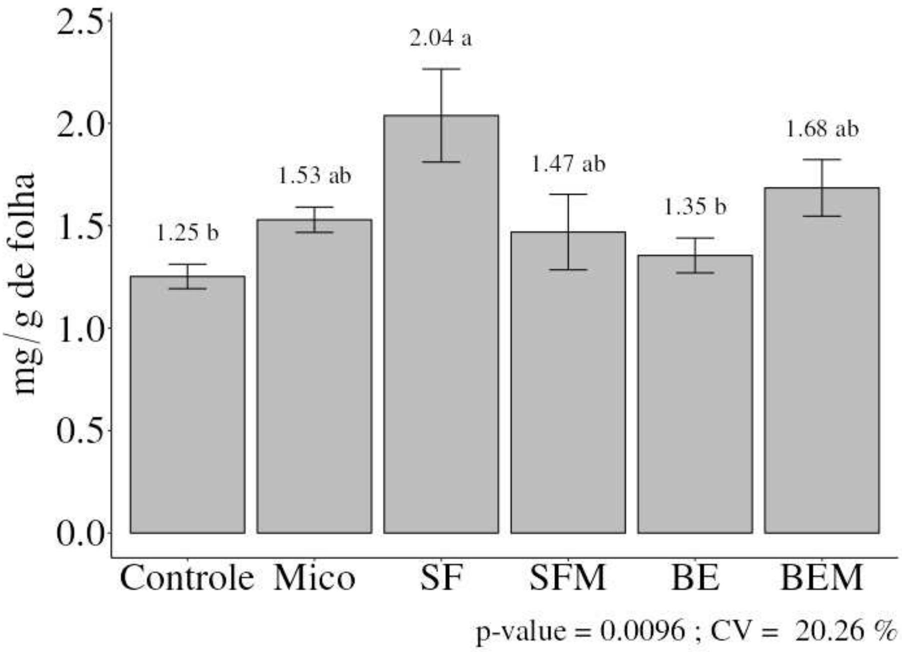
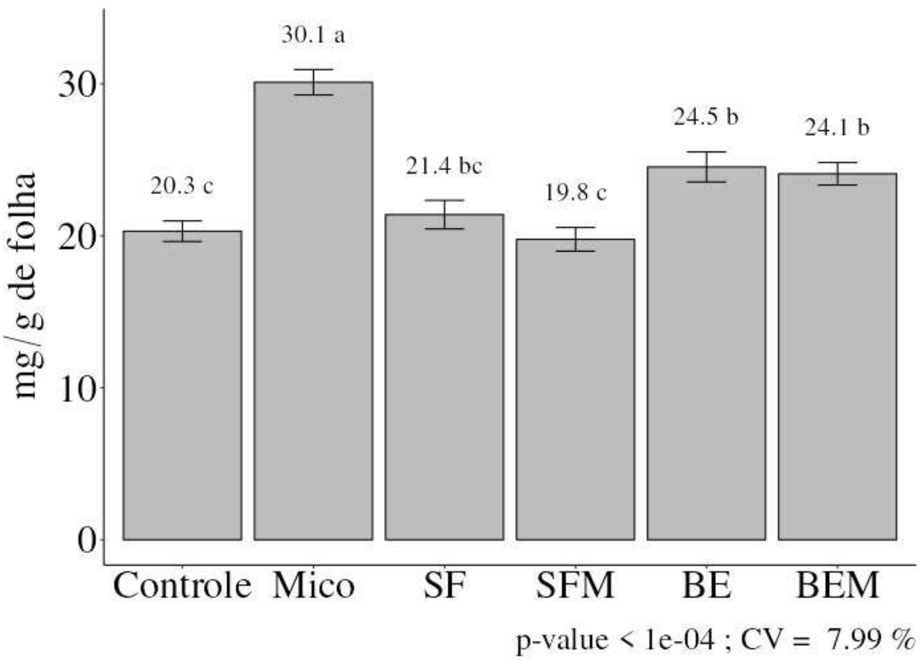


Figura 5. Concentração de nitrogênio (mg/g) das folhas de algodão, estimado após 45 dias de cultivo em casa de vegetação. Letras diferentes representam diferença significativa entre as médias.



4. DISCUSSÃO

Com o intuito de selecionar um isolado de cada origem, os isolados SF1 e BE1 foram selecionadas com base no potencial de multifuncionalidade demonstrada *in vitro,* as duas cepas apresentaram capacidade solubilizar fosfato, produzir AIA, sideróforos e EPS, sendo a produção de EPS fator crucial na seleção do isolado SF1. Enquanto para o isolado BE1, a capacidade de colonizar raízes *in vitro* foi um fator determinante. Dentro dessas características observamos diferentes mecanismos de promoção de crescimento, seja por aumento da disponibilidade de nutriente, estímulo do enraizamento ou proteção contra o estresse hídrico (Khoso et al., 2024). A utilização de cepas multifuncionais é uma das estratégias para aumentar a eficiências de produtos biológicos para a agricultura. Além disso, mecanismos como a solubilização de fosfato *in vitro* e a produção de AIA, já se demonstraram como bons parâmetros para seleção de bactérias promotoras de crescimento. Em estudo realizado com três bactérias capazes de solubilizar fosfato e produzir AIA (*Pseudomonas moraviensis*, *Bacillus safensis* e *Falsibacillus pallidus*), houve um aumento de até 14,42% na produtividade da cultura do trigo em campo e cerca de 122% de fósforo lábil no solo (Wang et al., 2022).

A capacidade de colonização radicular, processo pelo qual as células bacterianas se fixam na superfície da raiz e proliferam formando biofilmes, representa um mecanismo essencial para uma interação bem-sucedida entre planta e microrganismos (Nihorimbere et al., 2011). A avaliação realizada em raízes de tabaco não exclui a capacidade das demais cepas em colonizar raízes no solo, mas é um forte indicativo da alta capacidade de colonização da cepa BE1. Experimentos em casa de vegetação deverão ser realizados investigando a persistência e concentração dessas bactérias após inoculação, frente a competição com a microbiota nativa do solo.

A partir do sequenciamento do 16S rRNA, identificamos a cepa BE1 pertencente ao gênero *Micrococcus* sp. No geral, são amplamente difundidas na natureza, podendo até mesmo ser essenciais para manter o equilíbrio da comunidade microbiana natural da pele humana. Normalmente, não são patogênicas, mas algumas cepas de *M. luteus* são conhecidas como patógenos oportunistas para infecções nosocomiais capazes de causar bacteremia, pneumonia, endocardite, linfoma, artrite séptica e outras doenças (von Eiff et al., 1996; Ianniello et al., 2019;

Khan et al., 2019). Existem relatos da presença desse gênero bacteriano colonizando o interior de esporos micorrízicos (Bharadwaj et al., 2008). Trabalhos recentes têm demonstrado efeitos positivos da coinoculação de FMA e *Micrococcus* sp (Afrangan et al., 2023; de Carvalho Neta et al., 2024). A coinoculação de *Glomus versiforme* e *Micrococcus yunnanensis* demonstrou efeito sinérgico na redução do estresse salino em *Brassica napus* L. em casa de vegetação, melhorando consideravelmente a atividade enzimática antioxidante e o estado nutricional das plantas, aumentando a clorofila a e b, carotenoides, taxa de fotossíntese e porcentagem de óleo (Afrangan et al., 2023). A coinoculação de *R. clarus* e *Micrococcus endophyticus* foi capaz de aumentar a colonização micorrízica e promover o crescimento de milho sobre estresse salino (Carvalho Neta, de et al., 2024).

Identificada como *Lysinibacillus* sp., a cepa SF1 foi isolada da rizosfera de milho, o que é comum para bactérias desse gênero (Ercole et al., 2021). Diversos trabalhos já relataram a capacidade de cepas de *Lysinibcillis* sp. em solubilizar fosfato e produzir altos níveis de AIA *in vitro*, além da promoção de crescimento de plantas em casa de vegetação e campo (Naureen et al., 2017; Massucato et al., 2022; Pantoja-Guerra et al., 2023)

Quanto ao perfil de produção de ácidos orgânicos, foi detectado a presença de ácido glucônico e ácido málico. O primeiro é o ácido orgânico mais comumente associado a solubilização de fosfato. Produto da oxidação de glicose, assim como o ácido 2-ceto-gluconico (produto intermediário), ao longo dos anos vários trabalhos têm relacionado a produção de altos níveis desse ácido com a atividade de solubilização de fosfato *in vitro* e no solo (Hwangbo et al., 2003; Rodriguez et al., 2004; Stella and Halimi, 2015). Em relação ao ácido málico, apesar de não ser um dos principais ácidos relacionados a solubilização de fosfato, já foi relatada a detecção de altos níveis de produção desse ácido em avaliações *in vitro* de cepas solubilizadoras de fosfato de tricácio, fosfato de alumínio e fosfato de ferro (Gadagi, Shin e Sa, 2007; Kaur, Selvakumar e Upreti, 2021).

Detectamos a partir da quantificação, níveis significativos de produção de ácidos orgânicos pelas cepas SF1 e BE1. Os valores deste tipo de análise podem variar de acordo com as condições de fermentação, reagentes, metodologia e equipamento de quantificação, o que dificulta comparações entre trabalhos. Comparamos nossos dados ao detectado por Oliveira-Paiva et al. (2024), o qual avaliou cepas comerciais de bactérias solubilizadoras de fosfato disponíveis no mercado brasileiro (B*acillus megaterium* CNPMS B119 e *Bacillus subtilis* CNPMS B2084) com base na mesma metodologia utilizada neste trabalho. Tendo em consideração este contexto, podemos inferir que as cepas BE1 e SF1 apresentam uma produção de ácidos orgânicos relevante em meio contendo fosfato de tricálcio como fonte de P, cerca de 158,5 1 mmol L^{-1} e 182,1 1 mmol L^{-1}, frente a 57,16 1 mmol L^{-1} (CNPMS B119) e 40,64 1 mmol L^{-1} (CNPMS B2084) das cepas comerciais.

Com base na avaliação realizada em casa de vegetação na cultura do algodão, verificamos potencial na cepa SF1 em aumentar a disponibilidade de P para a planta, visto a média significativamente mais alta de P foliar em relação ao controle, atividade que é comumente relatada para cepas do gênero *Lysnibacillus* sp (Christian et al., 2019; Vitorino et al., 2024). Apesar das plantas inoculadas com SF1 e *R. clarus* (SFM) terem se desenvolvido significativamente melhor em relação ao controle, estes valores não diferiram em relação ao grupo Mico, o que indica que esses microrganismos não interagem de forma sinérgica (Anuar et al., 2023).

Em relação ao aumento na concentração de N em folhas de algodão tratadas com *Micrococcus* sp. BE1, bactérias desse gênero são importantes na ciclagem de nutrientes, produzindo enzimas líticas, também evidenciado pela atividade proteolítica em BE1 neste estudo. No solo, esse tipo de mecanismo pode significar uma maior disponibilidade de nutrientes como o N (Hou et al., 2017; Odu e Akujobi, 2012; Prakash Vimala and Basu, 2020). Um aumento nos níveis de N foliar também foi observado em plantas tratadas com FMA *R. clarus*, aspecto já descrito para este fungo, que além de ser um importante regulador da disponibilidade de P para a planta, também tem papel importante na obtenção de N (Veresoglou et al., 2012). Apesar disso, a coinoculação entre BE1 e *R. clarus* não demonstrou potencial sinérgico em relação a esse aspecto.

Apesar do efeito sobre a concentração de P e N foliar observado quando inoculadas as cepas bacterianas, não constatamos ganhos significativos de biomassa. Também não observamos atuação sinérgica entre as cepas bacterianas e o *R. clarus*. Porém, é possível que esse resultado possa ser observado em outras culturas, visto que a compatibilidade microrganismos hospedeiro tem alta influência nessa interação, além de fatores como a diversidade genética das bactérias presentes no ambiente, condições externas, como luminosidade, temperatura e a composição da matéria orgânica no solo (Meena et al., 2018).

5. CONCLUSÃO

Observamos um potencial da cepa *Lysinibacillus* SF1 em aumentar a concentração de P na planta. Porém, não foi constatado ganhos em biomassa para as plantas de algodão em relação ao grupo controle. Apesar das plantas inoculadas com SF1 e *R. clarus* (SFM) terem se desenvolvido significativamente melhor em relação ao controle, estes valores não diferiram em relação ao grupo Mico, o que indica que esses microrganismos não interagem de forma sinérgica ou antagônica. Quanto a cepa BE1, apesar de aumentar os níveis de N de folha, não demonstrou potencial na promoção de crescimento de plantas de algodão em casa de vegetação, seja inoculado de forma isolada ou em associação com *R. clarus,* também não apresentando interação significativa.

Com base nas análises *in vitro,* as duas cepas selecionados tem potencial na promoção de crescimento vegetal. Porém não constatamos ganhos significativos em relação a cultura do algodão em casa de vegetação. São necessários novos experimentos em casa de vegetação, visando avaliar esse aspecto. A inoculação em outras culturas deve ser considerada.

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