Review Article

Applied Strategies to Enhance Biofungicide Field Performance: Mechanistic Insights, Formulation Interventions, and Design Considerations

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Abstract
The inconsistent field performance of biofungicides remains a major barrier to their widespread adoption despite their ecological advantages over chemical fungicides. This review synthesizes key strategies for improving biofungicide efficacy under field conditions, including antagonist optimization, microbial consortia, and supplementation interventions. These approaches are evaluated based on their mechanistic basis, compatibility constraints, and practical feasibility. Comparative synthesis shows that biofungicide performance emerges from interactions among the microbial antagonists, the target phytopathogen, and the application environment, explaining why no single strategy is universally effective. Based on these insights, an integrated design framework is proposed that emphasizes the cost-to-performance ratio as a guiding principle for biofungicide development. This framework links biological efficacy with environmental adaptability and economic feasibility, providing practical guidance for the development of robust and sustainable biofungicide technologies.

Keywords: Biofungicide, Cost-performance ratio, Field efficacy, Formulation strategies, Microbial antagonists, Sustainable agriculture

1 Introduction

Achieving global food security remains a critical challenge as the world's population continues to rise and agricultural systems face increasing pressure to sustain productivity. With estimates suggesting that food production must double to meet future demand [1], there is a need to improve current agricultural outputs.

Microbial inoculants have emerged as a promising tool due to their ability to enhance plant growth, induce resistance, and suppress plant diseases [2], [3]. Among these functions, their ability to inhibit plant diseases is a point of interest. Fungal pathogens infect a wide range of crops across multiple developmental stages, which cause substantial pre- and post-harvest losses [4], [5]. Although chemical fungicides remain the primary means of disease control, their repeated use contributes to resistance development [6]. Biological fungicides or biofungicides offer a safer and more sustainable alternative, but their performance under field conditions is highly variable.

This variability arises from a combination of ecological, practical, and economic factors. Ecologically, biofungicide efficacy is strongly influenced by environmental fluctuations, pathogen diversity, and the dynamic interactions between target pathogens and antagonistic microbes [7]. From a practical standpoint, maintaining product efficacy often requires specialized formulation, stabilization, and storage strategies [1]. These additional requirements can increase production and handling costs, which in turn limit production, particularly among resource-constrained and small-scale growers.

To address these challenges, biofungicide development focuses on formulation type and production optimization [3], [8]–[11]. While recent studies emphasize economic feasibility by using low-cost carriers and substrates, they do not emphasize

field efficacy [12]. Similarly, the use of genetically modified biocontrol agents was introduced, but a critical evaluation of potential trade-offs and key factors to be considered is not highlighted [13].

While numerous strategies have been proposed to improve biofungicide performance, they are frequently evaluated independently, making cross-comparison and strategic alignment challenging. This review consolidates these approaches into a coherent framework that links mechanisms, performance limitations, and formulation design considerations. Such a synthesis provides a practical reference for guiding future biofungicide development and evaluation.

2 Strategies to Improve Biofungicide Biocontrol Efficacy

The primary active component of biofungicide is the microbial antagonist capable of suppressing the target phytopathogenic fungus. However, field efficacy depends not only on microbial survival but on the antagonist's ability to rapidly establish on the host surface, compete within complex microbial communities, tolerate environmental stresses, and sustain anti-fungal activity over time.

Strategies reported in the literature to enhance biofungicide efficacy can be broadly organized into two conceptual categories, as illustrated in Figure 1. The first category encompasses approaches that focus on optimizing the biological antagonist itself through genetic enhancement [13] and the development of microbial consortia that combine complementary mechanisms and improve ecological robustness [14], [15]. The second category involves supplementation-based strategies, which include the use of reduced-dose fungicides [16], chemical and biological inducers [17], and plant-derived metabolites [18], which aim to enhance host defenses or generate synergistic interactions with microbial antagonists.

Together, these approaches target different stages of the plant-microbe-pathogen interaction and provide a multi-layered framework for improving biofungicide efficacy under variable field conditions.

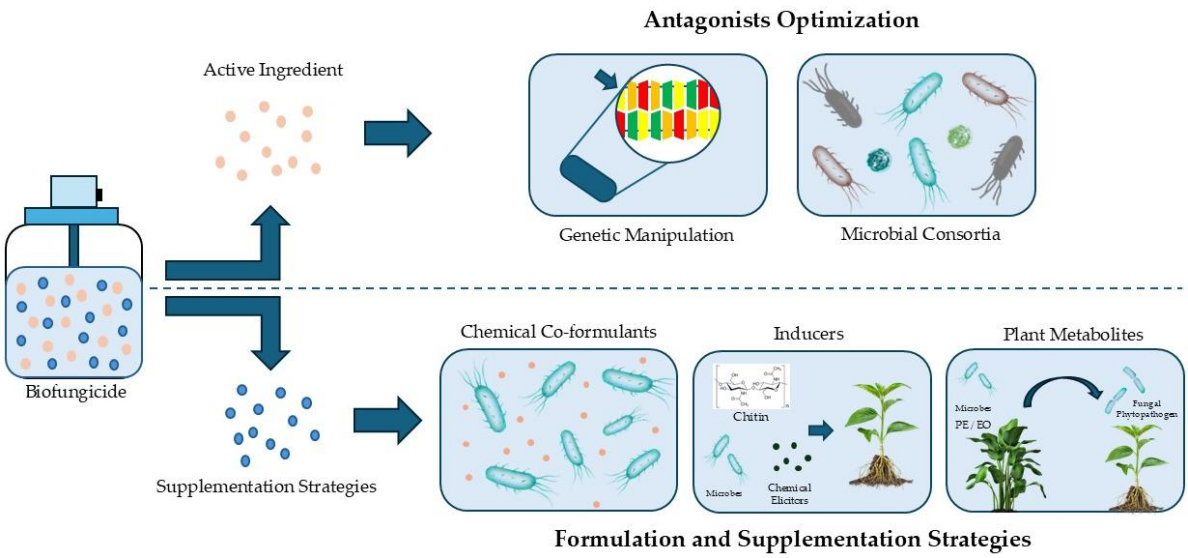

Figure 1: Overview of strategies to improve biofungicide biocontrol efficacy.

2.1 *Antagonist optimization*

Antagonist optimization aims to enhance the inherent biological performance of microbial agents to achieve more consistent disease suppression under field conditions. Key strategies include genetic enhancement to strengthen critical physiological or metabolic functions and the use of microbial consortia that combine complementary mechanisms to improve ecological resilience. Together, these approaches reinforce antagonist fitness against environmental and competitive stresses that commonly limit biofungicide efficacy.

2.1.1 Genetic manipulation

Genetic modification of the microbial antagonists provides a precise molecular-level strategy to

enhance biocontrol efficacy by strengthening traits directly associated with antifungal activity and field adaptability. As summarized in Table 1, most genetic manipulation efforts reported in the literature target genes involved in cell wall-degrading enzyme production, secondary metabolite biosynthesis, and stress tolerance, which collectively contribute to improved pathogen suppression under controlled and semi-field conditions.

Table 1: Antagonist optimization approach in biocontrol applications.

Category	Antagonist(s)	Strategy	Result	Target Pathogen	Disease	Ref.
			Fungal Antagonists			
Genetic Manipulation	*Trichoderma harzianum*	Overexpressing *chit33* gene	Enhanced extracellular chitinase activity and increased growth inhibition compared with wild type.	*Rhizoctonia solani*	Damping-off disease	[19]
	Trichoderma virens	Co-expression of β-1,3- and β-1,6-glucanase	Achieved up to 312% disease suppression under high pathogen pressure.	*Pythium ultimum* *Rhizoctonia solani*	Seed rot Damping-off	[20]
			Bacterial Antagonists			
Genetic Manipulation	*Pseudomonas protegens* Pf-5	Inactivation of the *retS* regulator	Increased production of 2,4-diacetylphloroglucinol (Phl), resulting in stronger antifungal activity.	*Rhizoctonia solani*	Damping-off	[21]
	Pseudomonas fluorescens SBW25	Knock-in of Phl-producing gene	*Phl* operon insertion combined with strong rhizosphere colonization suppressed fungal infection in pea without phytotoxic effects.	*P. ultimum*	Root rot	[22]
	Lactobacillus plantarum IMAU10014	Genome-shuffling	Improved antifungal activity by 192% to 200% after three rounds of genetic shuffling.	*Penicillium digitatum*	Root rot	[23]
	Streptomyces aureoverticillatus HN6	Mutagenesis	Mutant strain E3 exhibited the highest antifungal activity (72%); fermentation broths of E8, E87, and E95 completely inhibited fungal growth.	*Fusarium oxysporum*	Green mold	[24]
Consortia of Microorganisms	*Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus amyloliquefaciens*, *Trichoderma citrinoviride*	N/A	Consortia provided stronger fungal inhibition due to higher production of metabolites compared with single isolates.	*Macrophomina pheseolina* *Sclerotina sclero*	Charcoal rot White mold	[25]
	Trichoderma afroharzianum 5F, *P. fluorescens* 131B, *Bacillus licheniformis* 223B, *B. subtilis* 236B	N/A	Combined application achieved 76.5% disease control due to increased plant defense responses.	*Rhizoctonia bataticola*	Dry root rot	[26]
	B. amyloliquefaciens CECT 8238 and CECT 8237 *Pseudomonas chlororaphis* MA 342 *Pseudomonas azotoformans* F30A, *T. harzianum* T22 and ESALQ1306	N/A	Consortia effectively suppressed the pathogens across different bioassays via direct antagonism and induction of plant resistance following seed and foliar application.	*Fusarium oxysporum* *Botrytis cinerea*	Fusarium wilt Gray mold	[14]

Several studies demonstrate that overexpression or insertion of genes encoding chitinases, glucanases, and antibiotic biosynthetic operons leads to substantially higher inhibition of fungal phytopathogens compared to wild-type strains. However, despite these advances, genetically modified antagonists can exhibit fitness trade-offs, which are important to consider, as these may reduce field efficacy [27]. For example, low persistence in soil is observed for the improved strain of *P. fluorescens* SBW25 compared to its wild type [22]. While this can be considered a beneficial property, it may also reduce long-term biocontrol of the target phytopathogen.

Recent developments in genomics, including expanded microbial genome databases, integrated omics approaches, and precision tools such as CRISPR genome editing, have offered ways to fine-tune trait modifications with minimal off-target effects, potentially reducing ecological risk while improving specific biocontrol functions [28], [29].

Nevertheless, successful field deployment requires rigorous evaluation of genetic stability, potential horizontal gene transfer, persistence in agroecosystems, interactions with native microbiota, and compatibility with host plants [30]. The influence of local environmental factors should also be considered, as these influence biocontrol efficacy [31]. Aside from improvements in genetic operons related to biocontrol efficacy, microbial consortia offer unique responses and activities in the field.

2.1.2 Microbial consortia

Microbial consortia refer to the formulations composed of two or more microbial isolates of the same species or from different genera that work synergistically to suppress phytopathogens or promote plant growth [14], [32]. This approach aims to exploit the complementary mechanisms of multiple microorganisms to achieve a broader and more consistent spectrum of biocontrol activity by maintaining functional redundancy, as illustrated in Table 1.

Challenges remain in developing effective microbial consortia for biofungicide applications. Although combining multiple antagonists is conceptually attractive, more strains do not necessarily translate to stronger biocontrol [15], [33]. Similar interaction-dependent effects were observed in co-cultured *Trichoderma* strains, where mixed cultures produced higher levels of free amino acids than single strains [15].

Finally, a recurring trade-off exists wherein formulations optimized for plant growth promotion may not exhibit strong biocontrol activity, and vice versa [32]. In addition to this, the dominance of a single strain may dilute the intended functional balance, while differences in growth kinetics and stress tolerance can lead to uneven survival during storage and instability during large-scale co-fermentation. These findings underscore the importance of rigorous compatibility and balancing natural dynamics between microbes for developing robust, field-effective consortia-based biofungicides.

2.2 *Supplementation strategies*

While enhancing genetic traits, a consortium of antagonists focuses on the physiological state of the microbes involved. In contrast, supplementation strategies aim to give these microbes an advantage in their early phase of colonization. This is achieved by directly inhibiting the fungi with low doses of chemical fungicides, inducing systemic resistance in plants, and plant metabolites to inhibit the presence of native microbiota, as summarized in Table 2.

Table 2: Formulation supplementation approach in biocontrol applications.

Category	Antagonist(s)	Supplementation Strategy	Result	Target Pathogen	Disease	Ref.
			Fungal Antagonists			
Chemical Co-inoculant	*Trichoderma viride* CIAH240	Tridemefon, triophanate methyl, mancozeb, or alcidine	Combined treatment at 50 to 100 μg/g enhanced postharvest control, achieving >70% efficacy at low dose and >80% at higher dose.	*Alternaria alternata*	Alternaria fruit rot / Black rot	[34]
	Cryptococcus laurentii	Thiabendazole	Integrated treatment provided stronger and longer-lasting control of fungicide-resistant isolates in apple compared to single treatments.	*Botrytis cinerea*	Gray mold	[35]

Table 2: (Continued)

Category	Antagonist(s)	Supplementation Strategy	Result	Target Pathogen	Disease	Ref.
	Rhodosporidium kratochvilovae LS11; *Cryptococcus laurentii* LS28	Boscalid (BOSC) and cyprodinil (CYPR)	Integrated treatments with BCAs and fungicides reduced fruit rot by up to 98% after 7 days of storage.	*Penicillium expansum*	Blue mold	[36]
			Bacterial Antagonists			
Chemical Co-inoculant	*Pseudomonas fluorescens* Pf1	Supplementation of azoxytobin	Biocontrol agent combined with a half-rate fungicide dose achieved disease suppression comparable to a full fungicide dose.	*Colletotrichum capsica; Leveillula Taurica*	Anthracnose; Powdery mildew	[37]
Biological and Chemical Inducers	*Pseudomonas fluorescens*	Chitosan	Combined treatment with chitosan significantly increased peroxidase activity, indicating enhanced host defense response.	*Peronospora viciae*	Downy midlew	[38]
	Bacillus subtilis	Chitosan and Humic Acid	Integrated treatment increased tomato plant biomass and reduced disease incidence by 45.1%.	Wilt-causing pathogen	Vascular wilt	[39]
	Bacillus subtilis B4	Benzo-(1,2,3)-thidiazole-7-carbothioic acid S-methyl ester (BTH)	Cultivation with BTH increased the antagonist population fivefold, and when co-applied to seedlings, significantly reduced disease incidence compared to single treatments.	*Pectobacterium carotovorum* SCC1	Soft rot	[40]
	Bacillus subtilis B4	Acibenzolar-S-methyl (ASM)	Combined application of *B. subtilis* B4 and ASM significantly suppressed disease compared to individual treatments, with ASM also enhancing bacteria population during cultivation.	*Colletotrichum orbiculare*	Anthracnose	[41]
Plant Metabolites	*Bacillus amyloliquefaciens* HF-01	Tea Saponin	Combined treatment with tea saponin (50 μg/mL) achieved >90% control, comparable to chemical fungicide treatments.	*Penicillium digitatum; Penicillium italicum; Geotrichum candidum*	Green mold; Blue mold; Sour rot	[42]
	Bacillus spp. SS-12.6	Thyme and Savory Oil	Seed treatment with crude lipopeptide extract (CLE) reduced fungal infection by up to 85% while maintaining acceptable germination; combinations with essential oils showed additive effects.	*Fusarium* spp.	Fusarium rot / Damping-off	[43]
	Bacillus subtilis	Cumin, Cardamom, and Thyme Essential Oils	Combined application of *B. subtilis* and 2% cumin essential oil provided disease control comparable to chemical fungicides.	*Sclerotium cepivorum*	White rot	[44]

2.2.1 Chemical co-inoculant

One of the primary goals of biofungicide development is to reduce reliance on synthetic fungicides, and the strategic integration of fungicides at reduced doses can enhance field consistency and reliability [16]. In this approach, the rapid suppressive effectiveness of chemical fungicides complements the long-term antagonistic and immune-inducing actions of microbial agents.

The advantage in combination with fungicides, even in small dosages, is the ability of the

formulation to target and inhibit fungicide-resistant isolates in the field [35]. However, its successful integration depends on the sensitivity of the antagonist to the fungicide use. While *Pseudomonas fluorescens* is tolerant to azoxystrobin from 100 to 300 ppm, the presence of the dithiocarbamate fungicide at the recommended dose can inhibit *Bacillus cereus* C1L [37], [45].

In essence, chemical-biological co-formulations should be viewed as a transitional optimization strategy rather than opposing applications. The primary objective of this approach is to provide antagonists with a competitive advantage in their early colonization of the phyllosphere or rhizosphere. Alternatively, enhancements can work indirectly by priming the plant's own defense systems through biological and chemical inducers.

2.2.2 Biological and chemical inducers of resistance to plants

Plants possess innate defense systems that can be enhanced through biological and chemical inducers that activate immune pathways and stimulate the accumulation of defense enzymes and metabolites [17].

The first type of inducers are biological inducers that include beneficial microbes such as *Bacillus mycoides* and *Pseudomonas* spp., which trigger systemic resistance through the activation of defense enzymes like chitinase and β-1,3-glucanase [46], [47]. Their metabolites, such as indole-3-acetic acid, chitinase, siderophore, and lipopeptides, further stimulate resistance and enhance plant growth [48], [49]–[51]. Other types of biological inducers include natural polymers like chitin, chitosan, and their modified derivatives, which elicit reinforcement of the plant cell wall and activate defense genes [52].

The second type of inducers is chemical or synthetic-based, which activate plant immunity by mimicking or amplifying natural defense signals. An example of a chemical inducer is acibenzolar-S-methyl, a synthetic analogue of salicylic acid, which induces systemic acquired resistance in the absence of actual pathogen infection [53].

However, the effectiveness of inducers is highly context-dependent, varying across plant species and target pathogens. For instance, the use of laminarin, a β-1,3-glucan derived from brown algae, in combination with chitin only enhanced reactive oxygen species production in wheat, and is unable to significantly inhibit *Fusarium graminearum* compared to the use of chitin alone [54], [55]. In addition to this, the use of chitin and its related derivatives, such as chitosan, may induce phytotoxicity in plants at high concentrations [56].

Overall, both biological and chemical inducers complement each other in the management of plant diseases. Biological inducers offer environmental safety and persistence, while chemical inducers provide precise and rapid activation of defense pathways. Their combination can increase the biocontrol efficacy while also stimulating an increase in plant biomass. Beyond these inducers, plant-derived compounds can also provide an additional opportunity for boosting disease suppression and enhancing the integrated performance of biofungicide formulations.

2.2.3 Plant metabolites (plant extracts and essential oils)

Plant extracts (PE) and essential oils (EO) contain diverse bioactive compounds with confirmed antifungal activity [57]–[59]. For example, EO extracted from *Piper macedoi* Yunk. leaves effectively inhibit *Colletotrichum musae* at a concentration of 2.50 mL/L, performing comparably to the synthetic fungicide thiabendazole applied at 0.65 mL/L from a 485 g/L formulation [60]. Likewise, *Leonotis nepetifolia* extracts completely inhibited *Colletotrichum* spp. at a concentration of 2.5 mg/mL [61].

Although both originate from botanical sources, PEs and EOs differ substantially in composition, extraction method, and physicochemical properties. This difference influences their suitability and application for biofungicide formulations. PEs are commonly extracted by various solvents such as methanol, ethyl acetate, or water, with antifungal efficacy strongly influenced by solvent type and extraction conditions [57], [61]. In contrast, EOs are volatile compounds obtained primarily through a distillation process, such as hydro-distillation, which are energy-intensive and often yield relatively low quantities of oil [58], [60].

Despite their strong *in vitro* activity, pathogen sensitivity depends on the specific extract or EO profile, and effective suppression may require a higher concentration [58]. Increasing dosage, however, risks phytotoxicity, which varies among crops and developmental stages. To address these

limitations, integrating plant-derived compounds with microbial biocontrol agents has shown promising synergy. Trichoderma culture filtrates (10%) combined with *Calotropis procera* extract (15 mg/mL) significantly protected cantaloupe roots from *Fusarium oxysporum*, outperforming individual treatments [62]. Likewise, co-application of *Bacillus subtilis* TM3 with betel leaf extract reduced the severity of ear rot disease from 19.52% (control) to 6.19% [63]. In fact, integration with microbial agents can reduce the concentration of plant metabolites required for effective biocontrol [64], thereby lowering the risk of phytotoxicity and highlighting the importance of integrated biofungicide design.

3 Toward an Integrated Design Framework of Biofungicides

The diverse strategies presented address the inability of the antagonists to compete with the natural microbiome or have low metabolic activity due to sudden environmental stress. These highlight the multifaceted nature of improving biofungicides, as no strategy is universally effective across diverse host-pathogen-environment systems. Understanding various considerations, as summarized in Table 3, is then important to effectively design a biofungicide formulation that can maintain its efficacy in various environmental conditions.

Genetic enhancement strengthens inherent antagonistic traits and colonization fitness, microbial consortia provide ecological redundancy, and co-formulation with fungicides, inducers, and plant metabolites aims to reduce pathogen pressure and modulate plant defenses. Collectively, these approaches reflect a shift toward integrated systems optimization, in which microbial physiology, plant responses, environmental conditions, and formulation design are treated as interacting determinants of field performance, as shown in Figure 2.

The development process begins with the target fungal phytopathogen, its infection biology, survival strategy, and ecological niche. These traits determine which antagonists are most appropriate to use to effectively inhibit the fungi at all stages of their life cycle.

Table 3: Comparative summary of biofungicides' improvement strategies.

Strategy Approach	Functional Role in Biofungicide Design	Costs Considerations	Ecological Considerations	Formulation Considerations
		Antagonist optimization		
Genetic Improvement	Enhances intrinsic biocontrol traits (e.g., antifungal metabolite production) to increase reliability and potency of antagonists under variable field conditions.	High initial R&D and regulatory costs; potentially lower long-term production costs if traits are stable and reduce application frequency.	Requires careful assessment of ecological risks, including fitness trade-offs, persistence, and horizontal gene transfer; regulatory scrutiny may limit deployment.	Requires genetically stable constructs compatible with fermentation, storage, and field delivery; integration with formulations that maintain expression of target traits.
Microbial Consortia	Provides functional redundancy and complementary mechanisms to improve robustness across diverse pathogens and environments.	Moderate to high production costs due to strain compatibility testing, co-cultivation, and quality control.	Generally favorable ecological profile if composed of native or well-characterized strains; risks of interspecies competition or dominance shifts *in situ*.	Increased formulation complexity; challenges in maintaining balanced viability and stability of multiple strains during storage and application.
		Supplementation strategies		
Chemical co-inoculants	Offers rapid pathogen suppression during early colonization with microbial antagonists.	Reduced chemical input lowers costs relative to full fungicide programs; added formulation complexity may increase development costs.	Lower chemical loads reduce environmental impact, but ecological compatibility depends on fungicide type and dose.	Requires compatibility screening between antagonists and fungicides; optimized timing, sequencing, or co-formulation strategies are critical. Use of microbial metabolites or cell-free supernatants may be considered in combination with chemical co-inoculants.

Table 3: (Continued)

Strategy Approach	Functional Role in Biofungicide Design	Costs Considerations	Ecological Considerations	Formulation Considerations
Inducers	Primes plant immune systems (ISR/SAR), indirectly enhancing biofungicide performance and the plant's disease resistance.	Moderate recurring costs depending on the inducer's type and application frequency.	Generally low ecological risk, though plant- and context-specific responses may affect non-target interactions.	Must ensure stability and bioavailability of inducers in formulations; risk of phytotoxicity at high concentrations, which requires precise dosing.
Plant Extracts	Supplies multi-target antifungal and defense-priming compounds that complement microbial antagonists.	Variable costs depend on raw material availability and extraction efficiency; efficacy of plant extracts depends on the type of solvent, which may be costly for scaling up. Formulation complexity may increase production costs.	Renewable and eco-friendly, but dependent on sustainable sources of plant materials.	Batch variability and stability issues often require integration with microbes or carriers to reduce dosage and phytotoxicity. Compatibility with chosen antagonists, as it may inhibit the growth of antagonists in the field. Alternatively, the use of metabolites or cell-free supernatants may be considered.
Essential Oils	Delivers highly potent antifungal activity, particularly suited for postharvest or controlled environments.	High production costs and limited scalability restrict its use outside of high-value crops. Formulation complexity may increase production costs.	Low persistence reduces long-term ecological impact and limits field durability.	High volatility and oxidation may require encapsulation or emulsion-based systems for effective delivery. Compatibility with chosen antagonists, as it may inhibit the growth of antagonists in the field. Alternatively, the use of metabolites or cell-free supernatants may be considered.

The biological traits of the antagonists further constrain strategy selection. While supplementation with chemical fungicides can provide rapid suppression, some microbial antagonists are capable of degrading fungicides [65]–[67]. Although environmentally beneficial, this process may compromise biocontrol efficacy by diverting metabolic resources away from antifungal activity. In addition to this, the use of plant extracts and essential oils may also inhibit beneficial microbes. For instance, patchouli, clove, and lemongrass essential oils have antibacterial activity against *B. subtilis* [68]. This may prompt the use of cell-free supernatant or microbial metabolites with essential oils instead.

Among all factors that influence biofungicide performance, the environment serves as both the first limiting constraint and the primary determinant of strategy selection. Temperature, humidity, nutrient availability, and the composition of the native microbiome dictate whether an introduced antagonist can survive long enough to express its antifungal traits.

These same environmental conditions also shape the appropriate means of enhancing efficacy, as certain supplementation strategies, such as plant metabolites or essential oils, lose potency under high temperatures [69]. To increase the efficacy and stability of essential oils, they are often formulated as emulsions [70]. Likewise, the suitability of using single strains versus microbial consortia depends on the ecological stability of the application environment, as consortia offer redundancy in variable or stressful habitats but add complexity in stable systems. Environmental conditions also influence the proper timing and frequency of application to effectively increase its efficacy in the field [54], [71].

The final constraint that unified all these considerations is the cost-performance ratio, which ultimately determines whether any efficacy-enhancing strategy is practical beyond controlled experiments. Even highly effective antagonists, metabolites, inducers, or consortia lose relevance if their production or formulation requires inputs that

make field application economically unfeasible. Strategies such as essential oil supplementation, organic-solvent extracts, or genetically enhanced strains may offer strong antifungal activity, but their benefits must be weighed against scalability, resource intensity, and the marginal gains they provide under real farming conditions. Conversely, low-cost approaches, such as pairing aqueous plant extracts with microbial antagonists, may deliver moderate but economically acceptable improvements in disease suppression.

Thus, the cost-performance ratio serves as the final filter through which all strategies must pass, as it ensures that the selected enhancement not only improves biological efficacy but does so with a level of resource investment that supports adoption and sustained use in actual agriculture settings.

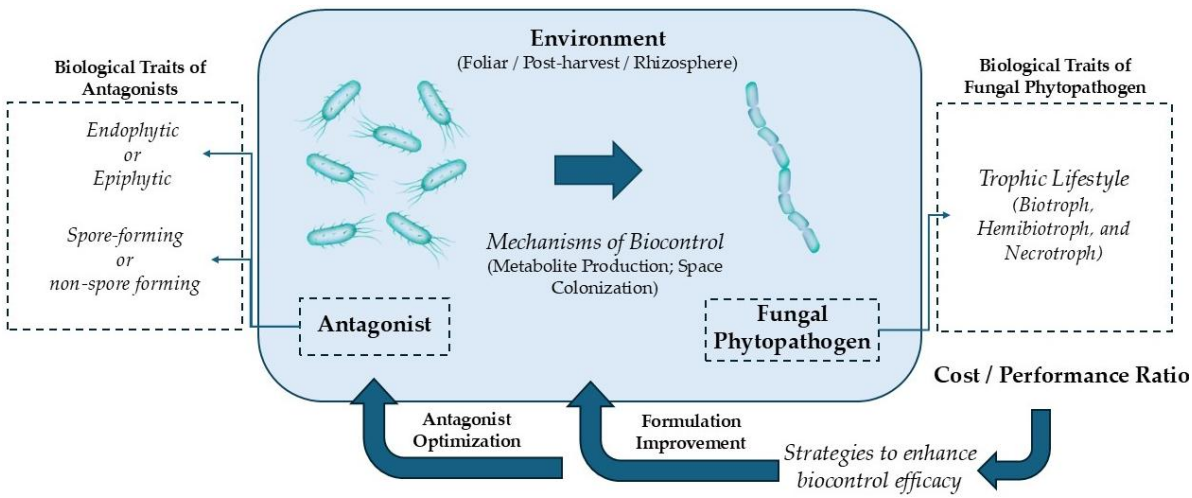

Figure 2: Conceptual framework for biofungicide formulation development.

4 Future Directions

Future research must move beyond single-strategy optimization and toward deliberate combinations of antagonist enhancement and formulation supplementation. While individual approaches address specific limitations in biological control, their integration introduces a new layer of complexity that remains insufficiently explored.

For antagonist optimization strategies, future studies should prioritize evaluating whether enhanced traits observed under controlled conditions persist across fluctuating field environments. In genetically modified antagonists, this includes assessing long-term ecological fitness, stability of introduced traits, and consistency of antifungal activity under abiotic stress. For microbial consortia, critical gaps remain in understanding strain compatibility over time, dominance shifts during storage and application, and the balance between functional redundancy and competitive exclusion under field conditions.

For supplementation strategies, on the other hand, should clarify how added chemical fungicides, resistance inducers, or plant-derived metabolites interact with microbial antagonists during early colonization and disease establishment. While integrated systems combining microbial agents, metabolites, and biopolymers, such as *Trichoderma afroharzianum* T22 and *Azotobacter chroococcum* 76A, and 6-pentyl-α-pyron (a *Trichoderma* secondary metabolite) with a carboxymethyl cellulose-based biopolymer to improve basil yield in greenhouse conditions [72], their performance under variable weather, their persistence after storage, and their stability in open-field environments remain largely unknown.

Beyond biological performance, cost-performance trade-offs represent a critical but underreported research gap. Many enhancement strategies increase formulation complexity through additional processing steps, specialized substrates, or multi-strain consortia, often improving efficacy, but their scalability, production cost, and marginal gains

under real farming conditions remain unclear. To illustrate this gap, microalgal extracts have potent antifungal activity and can enhance plant immunity; however, the associated costs in their production, despite being highly sustainable, limit their presence in the market for biofungicide use [73].

Future studies should therefore quantify not only the biological benefits but also the economic burdens that are associated with each strategy. This ties the strategy approach to increasing field efficacy with the production cost to determine market acceptability.

5 Conclusions

The shift from isolated experimental interventions to an integrated strategy for improving biofungicide efficacy reflects the essential interplay among four interdependent factors: the biology of the fungal phytopathogen, the physiological traits of the antagonists, the environmental context of application, and the suitability of supplementary enhancement approaches. Together, these dimensions determine whether efficacy is best improved through optimizing microbial traits, assembling complementary consortia, or introducing targeted supplementation such as inducers, metabolites, or reduced-dose fungicides. Importantly, the most successful solutions do not rely on maximizing any single component but on achieving a functional balance where biological performance aligns with practical manufacturability and field robustness. In this context, biofungicide development becomes not only a question of enhancing antifungal activity but of doing so at the lowest feasible cost while preserving ecological safety, stability, and resilience across diverse production and field environments.

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Author Contributions

K.C.T.C.: conceptualization, reviewing and editing, writing, editing, writing on original draft; M.V.T.: conceptualization; F.D.C.S.: reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors utilized the ChatGPT tool to enhance the language and readability of the manuscript.

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