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REVIEW ARTICLE

Exploration of bioagents tolerant to agrochemicals for better management of plant diseases

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ABSTRACT: The exploration of bioagents that are compatible with agrochemicals is required for advancing sustainable agriculture by improving disease management while reducing reliance on chemical inputs. Integrating bioagents with agrochemicals offers several advantages, such as enhanced disease control, decreased agrochemical usage, and minimized environmental impact. Additionally, this approach mitigates resistance development in pathogens by providing a complementary layer of protection. Practical applications of this integration include the development of co-formulated products, optimization of application timing, and the use of advanced delivery systems to maintain bioagent efficacy. Successful integration relies on thorough monitoring, adherence to regulatory standards, and ongoing innovation in formulation and application strategies. Ultimately, this exploration contributes to more sustainable and resilient agricultural systems by synergizing the strengths of bioagents and agrochemicals.

Keywords: Biocontrol agents, compatibility, fungicide, pathogen, resistance and sustainable agriculture.

INTRODUCTION

Today, feeding the increasing population demands efficient and sustainable agricultural practices which is a challenging aspect. The farmers are relying heavily on synthetic agrochemicals to manage plant diseases and prevent losses. The extensive use of agrochemicals has led to emergence of resistant pathogens and has residual effect on environment and human health. In this context, biocontrol agents (BCAs) have an alternative to conventional fungicides. Although BCAs put forward potential for disease management, their effectiveness can be limited by uncontrollable environmental factors. Combining BCAs with agrochemicals is expected to reduce fungicide usage and minimize residues on harvested crops. This strategy not only lowers the amount of fungicide but also employs diverse modes of action to reduce pathogen resistance, thereby mitigating risk of resistance development in agriculture (Ons *et al.,* 2020).

Integration of BCAs with agrochemicals represents a significant advancement in modern agriculture, devised in modernizing disease management strategies while fostering environmental sustainability. With challenges such as fungicide resistance, environmental degradation, and need for efficient resource use, it is crucial to explore BCAs that can work synergistically with agrochemicals. BCAs provide complementary means for managing diseases. When combined effectively with agrochemicals, BCAs can enhance control efficacy, decrease reliance on chemical, and reduce negative environmental impacts and vital in optimizing disease management strategies and advancing integrated disease management (IDM) practices that balance productivity with ecological stewardship. Successful integration depends on the progress in developing innovative formulations, optimization of application methods, and adherence to regulatory standards, eventually leading to more sustainable and resilient agricultural systems (Wojtkowiak *et al.,* 2006; Ons *et al.,* 2020).

Importance and need

BCAs act with various modes of action to defend plants from various pathogens. They act indirectly through plant resistance induction or directly by parasitism, antibiosis, or competition for nutrients and space. Combining BCAs with fungicides has been shown to enable a reduction in dose or frequency of fungicide applications, contributing to more sustainable agricultural practices and supporting marketing strategies that emphasize low or zero-residue produce.

BCAs must be compatible with fungicides, as fungicides can potentially inhibit their growth. When incompatibility is there, application strategies such as temporal separation, alternation, or spatial separation can be employed, though such separation is challenging for antagonists that directly target pathogens. Research should be precisely focus on upgrading fungicide resistant antagonists. Common biological resistance inducers combined with fungicides include *Trichoderma* sp. and *Bacillus* sp., offer long-lasting systemic effects that broaden disease control. While there is public concern about chemicals, those classified as Generally Recognized As Safe (GRAS) and chemical inducers of resistance are usually non-toxic to humans and the environment. Additionally, BCAs with long shelf-lives and stability offer significant advantages for supply chain and stock management.

BCAs compatible with agrochemicals

Understanding compatibility between BCAs and agrochemicals is essential in upgrading BCAs and integrate them in disease management. Combining of BCAs in field depends on how agrochemicals are employed for other diseases.

Trichoderma along with fludioxonil extensively improved control of *Fusarium* sp. and increased survival rates of coneflower seedlings in greenhouses (Wang *et al.*, 2005). Mancozeb was found extremely effectual in reducing *F. solani* mycelial growth and companionable with *T. harzianum* and *T. viride* at 0.05% and 0.1% concentrations (Singh and Varma, 2005). Integrating *T. asperellum* T8a with a low dose of captan provided greater *in vitro* growth inhibition of *Colletotrichum gloeosporioides* in mango (Peláez-Álvarez *et al.*, 2016). Combining fungicides with BCAs like *Trichoderma* spp. can produce synergistic effects. Terrero *et al.* (2018) verified compatibility of *Trichoderma* spp. with azoxystrobin and copper hydroxide fungicides. Ruano *et al.* (2018) applied *Trichoderma* spp. with fluazinam to control *Rosellinia necatrix* in avocado and improved root rot control. Palmieri *et al.* (2022) showed combining *Papiliotrema terrestris* and *B. subtilis* with synthetic fungicides resulted in 95.5–97% control in field and 63–91% in postharvest, with zero fungicide residues in fruit.

T. reesei C2A and *T. harzianum* with mancozeb improved mycoparasitic activity against *F. oxysporum*(González *et al.*, 2020; Huilgol *et al.*, 2022). Similarly, *T. asperellum* growth was supported by COC and mancozeb at 500 ppm (Maheshwary *et al.*, 2020). Tolerance of *Trichoderma* strains to agrochemicals is attributed to a variety of factors, including changes in oxidoreductase and ABC transporter genes, contributing to resistance against dichlorvos, mancozeb, thiram, tebuconazole, and carbendazim (Hirpara *et al.,* 2018; Sun *et al.,* 2019; Hu *et al.,* 2016). Compatibility of *Trichoderma*to fungicides was attributed to its membrane pumps and detoxification mechanisms (Ruocco *et al.*, 2009).

Rhodotorula mucilaginosa (Lv316) compatible with carbendazim, dimethomorph, mandipropamid, and azoxystrobin reduced disease incidence (Uribe-Gutierrez *et al.,* 2022). *R. mucilaginosa* showed no sensitivity to dimethomorph and mandipropamidthat target cellulose synthesis in oomycete membranes, affecting cell wall structure and spore germination. Mandipropamid may inhibit cellulose synthase-like PiCesA3 (Blum *et al.,* 2010). As yeast with chitin-based cell walls, *R. mucilaginosa* is not impacted by these fungicides (Bahmed *et al.,* 2003). Notably, *R. mucilaginosa* Lv316 exhibited high compatibility with carbendazim, which disrupts spindle formation during cell division in fungi (Yang *et al.,* 2011), and possibly will also restrain respiratory and fermentative metabolism in yeasts (Chiba *et al*., 1987). Combining *Pseudomonas fluorescens* 1, with azoxystrobin was more effective against of *Botrytis cinerea*, *Colletotrichum capsici*, and *Leveillula taurica*. *P. fluorescens*-16 showed compatibility with propiconazole, tebuconazole, trifloxystrobin + tebuconazole, azoxystrobin, carbendazim, and carbendazim + mancozeb, based on its growth. It was rated as good at 100 ppm concentration and was highly compatible with both azoxystrobin and carbendazim + mancozeb combination (Anand *et al.* 2010).

Carbendazim tolerant *Trichoderma harzianum* formulations were developed and tested effectively the management of groundnut root rot (Jalali *et al.* 2012). In Haryana, India, combining *Pseudomonas fluorescens*, *Mesorhizobium cicero*, and *Trichoderma harzianum* with carboxin and thiram resulted in reduced wilt incidence along with highest seed germination, increased grain yield in chickpea (Dubey *et al.,* 2015). In rice field, combination of *T. harzianum*, *P. fluorescens*, and carbendazim was found to be more effective against *Magnaporthe oryzae* than individual applications (Jambhulkar *et al.,* 2018). Additionally, *Piriformospora indica*, a root endophytic fungus, not only suppresses *Colletotrichum gloeosporioides* but also promotes plant growth and was compatible with strobilurins, triazoles, carbendazim, and pencycuron. Especially, germination of chlamydospores was significantly higher with these fungicides (Amrutha *et al.,* 2024). The more examples are mentioned in the Table1.

Copper induced Resistance in BCA

Bacillus subtilis applied with copper hydroxide (HCu) as part of an integrated strategy for citrus canker with active ingredients like cupric compounds shows potential consequences. Specifically, alternating applications of *B. subtilis* QST 713 with HCu significantly decreased incidence and severity of disease (Ibrahim *et al.,* 2016). In field, foliar applications of HCu achieved highest reduction in citrus canker incidence. A new copper sulfate formulation, Bioactive Copper (BioCu) includes amino acids and peptides and contains about 19% Cu (BAYER, 2016). The combination of *B. subtilis* and BioCu gave a 76% reduction in citrus canker under low disease incidence and up to 21.8% reduction under high disease incidence. Moreover, products with amino acids in their formulations have been shown to positively impact citrus tree height, leaf area, and both fresh and dry leaf mass (Mustafa and El-Shazly, 2015). Copper-based products can also trigger defense response in plants through post-formed biochemical factors. Treatment with BioCu, drastically amplified the *PR-2* gene 24 hours after application, with this elevated expression persisting for at least 7 days. This enhanced and prolonged gene activation in citrus trees treated with BioCu, compared to standard copper formulations, may be attributed to presence of complexing agents and amino acids in BioCu's formulation (Ramos *et al.,* 2022).

Table 1. List of compatible BCA with the agrochemicals

Biocontrol agent	Compatible agrochemical	Target disease	Reference
Fusarium oxysporum strain CS-20	Mefenoxam and mefenoxam + copper	Fusarium wilt of watermelon	Fravel *et al.* (2005)
Bacillus subtilis	Difenoconazole	Maydis leaf blight of corn (*Bipolaris maydis*)	Djaenuddin *et al.* (2021)
B. methylotrophicus TA-1	Fluopimomide	Gray mold in tomato	Ji *et al.* (2019)
F. solani	Thiophanate-methyl, fenhexamid, cyprodinil, boscalid and mancozeb	Fusarium crown and root rot disease in tomato.	Malandrakis *et al.* (2018)
Trichoderma asperellum	Mancozeb, Azoxystrobin, Cymoxinil+Mancozeb, Metalxyl+Mancozeb	*Pythium aphanidermatum, Pythium debaryanum, Sclerotium rolfsii* Sr1, *Sclerotium rolfsii* Sr3, *Fusarium oxysporum* f.sp. *lycopersici* and *Alternaria solani*	Manjunath *et al.* (2017)
Trichoderma spp.	Azoxystrobin and copper hydroxide	*Fusarium solani*	Terrero *et al.* (2018)
Trichoderma spp.	Fluazinam	*Rosellinia necatrix* in avocado	Ruano *et al.* (2018)
B. subtilis	Bioactive Copper (BioCu)	Citrus canker	Mustafa and El-Shazly, (2015)
T. aggressivum f. *europaeum*	Kresoxim-methyl, Pencycuron and Cymoxanil		Sánchez-Montesinos *et al.* (2021)
T. asperellum	Captan and Mancozeb	*F.solani*	Parraguirre Lezama *et al.* (2023)
T. asperelloides	Fosetyl Al, Amisulbrom and Cyflufenamid	Downy mildew and powdery mildew in grapes	Saha *et al.* (2023)

Piriformospora indica (Endophyte)	Strobilurins, strobilurins and triazoles, carbendazim and pencycuron	*Colletotrichum gloeospoiodes* causing anthracnos in yard long bean	Amrutha *et al.* (2024)
P. fluorescens 1	Propiconazole, tebuconazole, trifloxystrobin + tebuconazole, azoxystrobin, carbendazim, carbendazim + mancozeb	*Botrytis cinerea*, *Colletotrichum capsici*, and *Leveillula taurica*	Anand *et al.* (2010)
T. asperellum T8a	Captan	*Colletotrichum gloeosporioides* in mango	Peláez-Álvarez *et al.* (2016).
Rhodotorula mucilaginosa (Lv316)	Carbendazim, dimethomorph, mandipropamid, and azoxystrobin	Root rot	Uribe-Gutierrez *et al.* (2022)
B. subtilis QST 713	HCu	Citrus canker	Ibrahim *et al.* (2016)
Rhodosporidium kratochvilovae (Yeast)	Boscalid or cyprodinil	Blue mold caused by *Penicillium expansum*	Lima *et al.* (2011)
T. virens	Thiophanate-methyl	*Fusarium solani* and *Fusarium oxysporum* in dry bean	Abd-El-Khair *et al.* (2019)
B. megaterium	Carbendazim	*F. oxysporum* in tomato	Omar *et al.* (2006)
B. subtilis	Azoxystrobin	*Podosphaera xanthii* causing *Powdery mildew* on *zucchini*	Gilardi *et al.* (2008)
Combination of *P. fluorescens*, *Mesorhizobium cicero* and *T. harzianum*	Carboxin and thiram	*F. oxysporum* in chickpea	Dubey *et al.* (2015)
Clonostachys rosea	Prothioconazole	*F. graminearum and F. culmorum* in wheat and barley	Bengtsson, (2020)
T. asperellum	Copper oxychloride, Cymoxanil +Mancozeb, Mefenoxam+ Mancozeb and Cymoxanil +Famoxadone	Collar rot of elephant foot yam, tuber rot of cassava, stem and root rot of cassava, yam anthracnose and taro leaf blight	Veena *et al.* (2022)
B. subtilis	Tebuconazole	Rice false smut	Liu *et al.* (2023)
Clonostachys rosea	Fluxapyroxad and fluopyram a succinate dehydrogenase inhibitors (SDHI)	Tomato gray mold	Song *et al.* (2022)

Trichoderma asperellum SC012	Hymexazol	Fusarium wilt in cowpea	Zhang *et al.* (2021)
Yeasts and *Lactobacillus*	Mancozeb and Ridomil gold	Mango anthracnose caused by *Colletotrichum gloeosporoides*	Fenta and Kibret. (2021)
Trichoderma harzianum	Carbendazim	Fusarium wilt in carnation and marigold	Kumawat *et al.* (2019)

Multidrug resistant concept

Exploiting multidrug resistance (MDR) concept in BCAs provides a tactical improvement in IDM. By engineering or selecting BCAs with MDR traits, these agents be able to carry on and function effectively despite presence of multiple pesticides, fungicides, or herbicides. This competence helps in reliable disease control, even in face of chemical use that cab otherwise weaken efficiency. The MDR approach not only reduces need for excessive chemical use, promoting more sustainable agricultural practices, but also aids in managing resistance development in pathogens. By maintaining their biocontrol functions in chemically intensive environments, MDR BCAs contribute to more resilient and effective disease management systems, aligning with both productivity and environmental stewardship goals.

Techniques to develop agrochemical compatible BCA

Natural selection and breeding

The natural selection and breeding are important strategies in developing agrochemical compatible BCAs. Through the isolation and screening of naturally occurring strains with agrochemical exposure, identification of BCAs with inherent resistance traits can be exploited. The continuing exposure to agrochemicals in controlled surroundings promotes progression of tolerance, enabling assortment of robust isolates; however, once the selection pressure is withdrawn the tolerance to fungicide may come down. Hybridization of these robust isolates with other effective ones combines enviable traits, such as high biocontrol efficacy with agrochemical resistance. Recurrent selection and backcrossing improve these hybrids, escalating constancy and performance. Field trials make lawful effectiveness of these bred BCAs, ensuring compatibility with agrochemicals and their overall role in disease control.

Mutagenesis

The BCAs improvement for *Trichoderma* sp. holds substantial potential, with prime focus on developing fungicide tolerant mutants, along with improved hydrolytic enzyme production. Developing new molecules needs huge investment and modern agriculture will more and more depends on BCA. With climate change, there is increasing need for microbial pesticides that withstands abiotic stresses with improved biocontrol abilities. While genetic engineering offer opportunity of creating novel strains and likelihood of these GMOs overcome regulatory barriers and being approved for field use remains low. As a result, mutation will hold decisive role in developing superior strains of *Trichoderma* and facilitating their approval as feasible alternative to chemicals.

Several *Trichoderma* strains have been developed using mutagenesis to boost biocontrol properties. UV ray mutagenesis improved *Trichoderma* sp. mutants to carbendazim tolerance which strongly inhibits *Trichoderma* with remarkable variations compared to wild-type strains in appearance, growth habits, soil survival, antibiosis, and disease control efficacy (Papavizas and Lewis, 1983). *Trichoderma* sp. exposed to nitrosoguanidine and mutants selected on benomyl with superior rhizosphere colonization and biocontrol potential (Ahmad and Baker, 1988). Through a two-step mutagenesis progression through UV and gamma radiation, Mukherjee *et al.* (1999) developed stable benomyl tolerant mutants of *T. pseudokoningii* having better biocontrol capabilities compared to wild ones. Through gamma-ray, benomyl-resistant mutants with enhanced mycoparasitic activity of *T. virens* with distinct colony morphology, increased production of secondary metabolites such as the antimicrobial viridin, and improved disease control potential was done (Olejnikova *et al.* (2010).

Genome shuffling (GS)

Genome shuffling (GS) is a noteworthy progression in combinatorial engineering, first introduced by Stemmer group in 2002. This method includes *in vitro* homologous recombination of pool of preferred mutant genes through random fragmentation (Zhang *et al.,* 2002). Over traditional methods like mutagenesis and protoplast fusion, GS gives better competence for phenotypic improvement. The GS has accelerated strain upgrading processes through the recursive protoplast fusion between multiple parent strains, providing more hybrid strains. This approach allows for integration of advantageous traits from multiple parents, to achieve desired one in shorter period. Remarkably, two rounds of GS can bring about results that formerly necessary up to 20 years *via* classical improvement methods (Zhang *et al.,* 2002; Gong *et al.,* 2009).GS is multipurpose and not constrained to microbes with well characterized genetic backgrounds. It is a cost effective method without expensive facilities; a round of GS is comparable in cost to a cycle of protoplast fusion. The process is fairly uncomplicated and can be employed in most laboratories, which rely on protoplast fusion without classified as genetically modified (Zhang *et al.,* 2002). This peculiarity enables it to keep away from public concern frequently allied with GMOs (Gong *et al.,* 2009; Côrtes *et al.,* 2021).

Genome editing

CRISPR-Cas technology offers an influential tool for enhancing efficiency of BCAs by editing their genomes to get better resistance to specific fungicides. By employing CRISPR-Cas technology, researchers can bring in or boost resistance mechanisms within BCAs. Genome editing can be used to modify BCAs' metabolic pathways, allowing them to evade or counterbalance the fungicide's effects. CRISPR-Cas allows for defined genome editing, ensuring modifications are precise and do not upset other critical genes affecting BCAs role or compatibility with fungicides. Additionally, gene editing can design and construct synthetic metabolic pathways within BCAs to augment their ability to degrade or resist agrochemicals. The use of CRISPR in developing BCAs is subject to regulatory inquiry. Developing BCAs with resistance to fungicides must be cautiously managed to keep away from the potential for resistance development in pathogens as well.

Exploration of fungicide resistance mechanism

Fungicides are requisite for high value crops. Resistance to site specific fungicides has predominantly been associated to target and non-target site mechanisms. These mechanisms change the structure or expression, disturbing fungicide efficiency and resulting in diverse and varying resistance levels exploited in developing agrochemical compatible BCAs.

Alterations of target site

Mutations correspond to abrupt inheritable changes in DNA, impacting an organism's response to fungicides. Non-synonymous mutations, outcomes in amino acid substitutions at target site of a fungicide, repeatedly lessen binding affinity of fungicide to its target enzyme. The genetic mutations are decisive in development of fungicide resistance. Resistance to site specific fungicides like Quinone Outside Inhibitors (QoIs) can come up from a single point mutation in gene encoding target enzyme, imparts high-level resistance. QoI disrupts ATP production through disturbing electron transfer system, leading to demise of susceptible ones. However, mutations in cyt b alter binding site, preventing QoI from attaching to target protein and allows ATP synthesis in resistant ones (di Rago *et al.,* 1989; Chopra *et al.,* 2003).

On contrary, quantitative resistance have multiple genes providing resistance and typically develops progressively over time. This can occur with multisite fungicides or some single site fungicides. Fungi may develop quantitative resistance to demethylation inhibitors (DMIs) due to mutations in CYP51 gene, enhanced expression of target site with altered efflux pump action, or improved detoxification of fungicides (Hawkins and Fraaije, 2018). Reports frequently highlight point mutations at target sites linked to fungicide resistance. Organizations like Fungicide Resistance Action Committee (FRAC) and European and Mediterranean Plant Protection Organization (EPPO) make available comprehensive information on pathogen resistance to various fungicide classes, focusing chiefly on point mutations linked with QoIs, MBCs, DMIs, and SDHIs.

Target site overexpression

Resistance to DMIs and MBCs (methyl benzimidazole carbamates), is commonly allied with overexpression of target genes. For DMIs, resistance mechanisms frequently entail with enhanced expression of sterol 14 alpha-demethylase gene CYP51. In *Zymoseptoria tritici*, high levels of resistance were associated with insertions in CYP51 gene promoter region (Lucas *et al.,* 2015), in *Cercospora beticola* of beet and *Monilinia fructicola* of peaches, attributed to overexpression of CYP51 gene (Luo and Schnabel, 2008) while in *Pyrenophora teres* on oats, specific mutation (F489L) in CYP51A gene provides overexpression and gives resistance to DMIs. This overexpression subjected to genetic rearrangements or mutations in promoter region. *Mycosphaerella fijiensis* in bananas comprises parallel resistance patterns with changes in PfCYP51 gene promoter region show the way to heightened DMI resistance (Leroux *et al.,* 2007). This mechanism of overexpression observed in *Aspergillus flavus*, *A. niger*, *A. parasiticus*, and *Pyricularia oryzae* (Yan *et al.,* 2011; Fan *et al.,* 2013) but *C. gloeosporioides* shows DMI resistance through both mutations and overexpression of CYP51. In MBCs, resistance in *C. acutatum* in grapes is associated with overexpression of β-tubulin gene CaTUB1, regulated by CaBEN1 (Wei *et al.,* 2020; Sun *et al.,* 2013; Nakaune and Nakano, 2007). Overexpression of target genes remains a critical factor in developing resistance mechanisms against various fungicide classes.

Non-target site mechanisms

These mechanisms allow fungi to resist fungicides without changing their interaction with target site. Key non-target site resistance mechanisms comprise drug efflux transporters, CYP51 paralogs, mitochondrial heteroplasmy, alternative respiration pathways, altered sterol metabolism, detoxification processes, stress response regulation, quantitative resistance effects, and transcription factors (TFs). Resistance to DMIs is predominantly diverse and often associated with a variety of non-target site mechanisms. Key non-target site mechanisms comprise overexpression of drug efflux pumps, improved detoxification, and CYP51 paralogs existence. A noteworthy terror with non-target site resistance is its ability to award cross-resistance to a variety of fungicides with varied modes of action. This occurs because resistance mechanism affected fungicide detoxification or transport pathways rather than fungicide's target protein. Thus, non-target site resistance might comprise a negligible impact on fungal fitness, allowing resistant populations to persist and spread even in absence of fungicides. This poses a confront for fungicide management and underscores necessitate for developing efficient IDM strategies (Dorigan *et al.,* 2023).

In developing agrochemical companionable BCAs, understanding non-target site resistance mechanisms might leveraged to add on efficacy and robustness of BCAs. By targeting or bypassing these resistance mechanisms, researchers can design BCAs that remain effective against resistant fungal populations. This approach not only improves disease control but also integrates BCAs more effectively into offered agricultural practices, contributing to IDM and reduced reliance on chemical fungicides.

CONCLUSION

In conclusion, looking at compatible BCAs with agrochemicals is imperative in advancing modern agriculture. As reliance on synthetic agrochemicals intensifies, the associated risks of resistance development, environmental impact, and human health concerns highlights urge for alternative or supplementary management strategies. Effectual integration requires an inclusive understanding of interactions between BCAs and agrochemicals to optimize their joint effectiveness while preventing downbeat impacts on BCAs performance. By exploring and developing compatible BCAs, expansion in disease management systems can be seen, delays resistance development, and promote sustainable agricultural practices. The research should also further focus on fungicide resistant antagonists and their bypass mechanism to exploit it in various ways. The Central Insecticide Board (CIB) and FRAC have to look into the concept and frame guidelines for the proper implementation. Thus, compatibility of BCAs with agrochemicals will help in IDM.

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