GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(Review Article)

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The effects of interaction between *Trichoderma* Fungi and Arbuscular Mycorrhizal Fungi on protect and improve the growth of plants

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GSC Biological and Pharmaceutical Sciences, 2024, 29(03), 276-285

Publication history: Received on 16 November 2024; revised on 26 December 2024; accepted on 28 December 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.29.3.0488

Abstract

The adding of biofertilizer on agriculture crops become essential and very important options to reduce the adverse effects on environment and human healthy via using the fungicides. So, the beneficial microorganisms such as *Trichoderma* fungi and Arbuscular mycorrhizal fungi have ability to colonization the region near plant roots thus provide save place to plants and promoting plant growth and biocontrol the diseases around plant roots. *Trichoderma* and AMF have many mechanisms that play role to enhance growth of plants some of these mechanisms' similarity in work and other its unique to either *Tricoderma* or AMF. So, in this review exhibited the positive effects of interaction between *Trichoderma* and AMF and important mechanisms that using by them. We conclude from result of many studies that the synergistic interaction between *Trichoderma* and AMF is considered the promise options to application in sustainable agriculture.

Keywords: Trichoderma; Arbuscular fungi; Biocontrol agents, growth, AMF

1. Introduction

Trichoderma and arbuscular mycorrhizal fungi (AMF) are beneficial soil microorganisms that can enhance plant growth, disease resistance, and stress tolerance. Trichoderma species interact directly with plant roots, promoting growth and inducing systemic resistance against pathogens [1]. AMF improve nutrient uptake and root development, while both fungi act as elicitors of plant stress responses and secondary metabolite production [2]. *Trichoderma* strains can induce tolerance to abiotic stresses like drought and salinity by enhancing root growth, nutrient uptake, and protection against oxidative stress and *Trichoderma* have ability to produces bioactive compounds that stimulate plant growth and induce systemic resistance [3]. The combination of AMF and *Trichoderma* can have synergistic effects on disease control, as demonstrated in melon plants against *Fusarium* wilt. However, the effectiveness of this interaction depends on the specific AMF species used, with Glomus intraradices showing the most promising results when combined with *Trichoderma harzianum* [4].

Arbuscular mycorrhizal fungi (AMF) and *Trichoderma* spp. form symbiotic relationships with plants, enhancing their growth and stress tolerance. AMF improve nutrient uptake, especially phosphorus, while receiving plant carbohydrates [5]. Both symbionts help plants cope with abiotic stresses like drought, salinity, and heavy metals by improving nutrient uptake, regulating genes, and enhancing antioxidant production. They also protect plants against pathogens through competition and improved defense mechanisms. However, intensive agricultural practices can hinder AMF community formation [5],[6]. *Trichoderma* genes have been successfully transferred to plants, enhancing stress tolerance and

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bioremediation capabilities [7]. However, Overall, AMF and *Trichoderma* play crucial roles in improving plant-soil interactions and ecosystem stability.

The combined application of *Trichoderma harzianum* and Aarbuscular mycorrhizal fungi (AMF) has shown promising results in enhancing plant growth and disease resistance. Studies have demonstrated that this combination significantly improves plant productivity in various species, including Brassicaceae plants [8]. tomatoes [9]. maize [10]. and tea [11]. (Wanjiru, 2009). The synergistic effect of *Trichoderma harzianum*and AMF leads to increased root colonization, improved nutrient uptake, and enhanced plant growth parameters such as shoot and root dry weights. Additionally, this combination has shown potential in reducing disease severity, particularly against *Fusarium* wilt in tomatoes. The interaction between *Trichoderma harzianum*and AMF also modulates the composition of native soil and rhizosphere microbiota, which may contribute to their beneficial effects on plant growth and health [9]-[11].

1.1. Mechanisms of interactions (*Trichoderma* spp)

1.1.1. Biocontrol agent

Trichoderma species are widely recognized as effective biocontrol agents against various plant pathogens, particularly soil-borne fungi. These fungi exhibit antagonistic activities through mechanisms such as mycoparasitism, antibiosis, and competition for nutrients. *Trichoderma* spp. produce mycolytic enzymes and antifungal metabolites that inhibit pathogen growth fungi. Trichoderma-based biocontrol products are commercially available worldwide as alternatives to chemical pesticide These fungi are considered eco-friendly and cost-effective options for sustainable disease management in agricultures [12] – [14].

The fungal species *Trichoderma* has worldwide occurrence and can be easily isolated from a variety of soils, decomposing woods and other sporocarps. The potentiality of different *Trichoderma* species as effective biocontrol agents against plant diseases especially those caused by soil borne pathogens have been demonstrated long ago. They directly influence the growth and development of mycelia or other surviving propagules of pathogenic fungi through mycoparasitism commonly by releasing antimicrobial secondary metabolites, secreting enzymes that degrade fungal cell wall and forming structures aimed to restrict pathogen growth. Among the fungal biocontrol agents, the significance of *Trichoderma* is very high due to its mycoparasitic potentiality against a wide range of fungal pathogens such as *Botrytis cinerea*, *Fusarium spp*., Pythium spp., *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Sclerotinia sclerotiorum*. *Trichoderma* strains are being used as an alternative to chemical pesticides to manage various plant pathogens mostly attributed to their mycolytic and antibiosis activities as well as plant host mediated physiological changes [15].

1.1.2. Trichoerma as Plant growth promotion

Trichoderma species are versatile fungi that promote plant growth through various mechanisms. They colonize plant roots, enhancing nutrient uptake and mineral mobilization. These fungi also induce systemic resistance against pathogens and mitigate abiotic stress effects through antioxidant production [16]. *Trichoderma* produces growth-promoting compounds, including indole acetic acid and volatile organic compounds, which stimulate plant development [17]. Studies have shown *Trichoderma's* effectiveness in improving growth and yield in various crops, such as lettuce, radish, and quinoa. The use of Trichoderma-derived secondary metabolites offers a promising, environmentally friendly alternative to chemical fertilizers, particularly beneficial for small-scale farmers in challenging environments like the Andean highlands [18]. Additionally, *Trichoderma* can act as a bio-pesticide, fertilizer, and biostimulant in agriculture [19].

The use of bio-fertilizer is getting very popular since it enhances the growth, development and crop yield by supplying and increasing the nitrogen (N) availability and by producing certain substances like auxin, cytokinin and gibberellins, which are helpful in the growth of plants. [20]. *T.harzianum* improved growth the tomato seedling by increase nutrient element , uptake and permit microbial colonization [21]. *Trichoderma* fungus have ability to promoting plant growth via increased nutrient uptake and to protect them against biotic and abiotic stresses [22]. *Trichoderma* spp exhibited high efficacy as biofertilizer, increasing the nutrients solubility and uptake by crops when applied to soil, on seeds or leaf surfaces. An earlier report showed the correlation between increased root growth induced by *Trichoderma* fungus and shoot biomass production in maize. *Trichoderma harzianum* increased shoot morphological parameters and fresh and dry weight in tomato seedlings due to up-regulated Ca, Mg, P and K accumulation. *Trichderma* fungus applied in line with fertilizers increased growth of tomatoes that may lead to reduction in fertilizer dosages and minimizing their negative interactions in agricultural ecosystems. [2].

1.1.3. Induced systemic resistance (ISR).

Trichoderma species are effective biocontrol agents that can combat various plant pathogens and provide numerous benefits to plants. Trichoderma's capabilities extend beyond disease control, including ameliorating abiotic stresses, improving nutrient uptake, increasing nitrogen-use efficiency, and enhancing photosynthetic efficiency. These effects are achieved through the reprogramming of plant gene expression, likely by activating specific plant pathways. [23]-[24]. These fungi induce systemic resistance in plants, enhancing their defense mechanisms against a broad spectrum of pathogens. The induced systemic resistance involves jasmonic acid and ethylene signaling pathways, leading to the expression of defense-related genes. [25]. The induced systemic resistance involves jasmonic acid and ethylene signaling pathways, leading to the expression of defense-related genes. *Trichoderma* species are increasingly used in agriculture for both disease control and yield improvement, making them valuable tools for sustainable crop production [26].

Species of *Trichoderma* have the ability to combat numerous foliar, root, and fruit pathogens and even invertebrates such as nematodes. They also have many other capabilities such as ameliorating abiotic stresses, alleviating physiological stresses, enhancing nutrient uptake in plants, increasing nitrogen-use efficiency in crops, and improving photosynthetic efficiency. All of these capabilities are a result of their abilities to reprogram plant gene expression, may be through activation of a limited number of general plant pathways.[27]. Interactions between *Trichderma* spp and plant roots involve recognition attachment, penetration, colonization and nutrient transfer from roots. Any external interaction with roots triggered immune responses in plants due to the recognition of microbes as pathogens through the perception of microbe-associated molecular patterns by plants. However, *Trichoderma* spp manipulated the plant immune system by remodeling their transcriptome and proteome, thus TR were recognized as nonpathogenic. *Trichderma* fungus were able to induce jasmonic acid (JA) and ethylene (ET) synthesis, whose molecules are involved in the development of induced systemic resistance (ISR). Among substances produced by *Trichderma* spp are also volatile and nonvolatile compounds, such as 6-n-pentyl-6H-pyran-2-one gliotoxin, viridian, harzianopyridone, harziandione and peptaibols. Seedlings of Arabidopsis thaliana treated with volatile organic compounds released by *Trichoderma* spp had enhanced biomass production, root branching and flowering [2]. Isolates of several other *Trichoderma* spp. have been demonstrated that these isolates can reduce the severity of foliar diseases, presumably by inducing systemic resistance in plants [28].

1.2. Mechanisms of interactions (AMF)

1.2.1. Nutrient uptakes

Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with plants, enhancing nutrient uptake and providing non-nutritional benefits). they also contribute to improved water uptake, disease resistance, and soil aggregation, highlighting the multifaceted nature of this symbiotic relationship [29]. AMF improve plant acquisition of phosphorus, nitrogen, and other nutrients through an extensive hyphal network and specialized transporters The symbiosis involves complex mechanisms of mutual recognition and bidirectional transport of nutrients and organic compounds through specialized interfaces within host root cells. [30], [31]. The efficiency of nutrient transfer is influenced by soil conditions, particularly phosphorus and zinc levels. AMF colonization significantly affects the uptake of both macro- and micronutrients, with the most substantial benefits observed under nutrient-deficient conditions [32]. Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with most terrestrial vascular plants, enhancing nutrient uptake, particularly phosphorus. The extensive hyphal network of AMF can access fine soil pores unreachable by root hairs, significantly improving nutrient uptake. [33]-[34]. This symbiosis benefits both partners: plants provide photosynthates to fungi, while AMF improve water and nutrient absorption. Additionally, AMF enhance plant tolerance to various abiotic stresses, including drought, salinity, and heavy metals [6],[35].

1.2.2. Stress tolerance

The Arbuscular mycorrhiza fungi has ability to improves plant tolerance to drought by production the phytohormones. hormone homeostasis regulates plant tolerance against water stresses. The abscisic acid (ABA) that induce stomatal closure and reduces cell water loss. Also, the jasmonic acid (JA) in interacts with (ABA) to regulate plant response to water stress. also, mechanisms are involved in plant tolerance to drought. the inoculation with AMF induces the produce the phytohormones strigolactone and auxin that involved in plant water stress regulation. plants under salinity medium produce excessive amount of reactive oxygen species (ROS) which consider harm plant growth. So the adding inoculum of Arbuscualr mycorrhizal fungi to these plants demonstrated the reduce the deleterious effects This is mainly related to a combination of biochemical, physiological, and nutritional ects . Among the mechanisms involved in salinity tolerance in AMF inoculated plants, we have the enhancement of water absorption capacity and nutrient uptake, the accumulation of osmoregulators like proline and sugars. The ionic homeostasis and the reduction in Na+ and Cl- uptake, in addition, it has been demonstrated that AMF colonization enhances stomatal conductance and reduces the oxidative damage in plants exposed to salinity [5]. Inoculation of Arbuscular mycorrhiza fungi for plants under salinity stress led to Improved growth, higher antioxidant enzyme activity, elevated proline and phenolic content, and improved uptake of vital mineral elements have been observed. Addition, reduce the absorption of sodium ions [36].

1.2.3. Soil structure improvement

The soil is supply essential the ecosystem services that relate to provide better crops for consumers. The structure of soil is long been known to be influenced by the soil physical, chemical and biological properties. and that these properties interact to form stable aggregates in soil. application of Arbsucular mycorrhizal fungi to soil improve the soil water retention and soil structural stability [37]. The arbuscular mycorrhizal fungi mycelia colonization the rhizosphere near the root of plants its benefits from sugar in host roots to its survive and return it provide for plants the most important minerals such as P, K and N that essential for plant growth and functions. The mycelia and exudation of arbusular mycorrhizal fungi in soil produce the Glomalin and involved the organic acids together that protect soil from erosion and improved soil structure and carbon sequestration and enhance soil microorganism's activities [38]. AMF can directly influence bacterial communities via the deposition of mycelium products that serve as substrates for bacterial growth. AMF exudates influence the abundance and activities of specific fungal and bacterial species. Interestingly, bacteria (such as *Paenibacillus spp*.) have recently been isolated from AMF mycelia that has roles in enhanced the soil aggregation [39].

1.2.4. Interaction between Trichoderma and AMF for biocontrol agents.

The interaction between arbuscular mycorrhizal fungi (AMF) and *Trichoderma* species has shown promising results in biocontrol of soil-borne pathogens. Studies have demonstrated that dual inoculation of AMF and *Trichoderma* can enhance plant growth, reduce disease severity, and improve defense-related physiological and biochemical activities in various crops. Combined application of *Trichoderma harzianum* and AMF demonstrated synergistic effects in suppressing *Fusarium* wilt in melon plants [4]. and tomatoes. [40]. This combination also effectively reduced damping-off disease caused by *Rhizoctonia solani* in tomatoes, while enhancing phosphorus uptake and plant growth [41]. In pigeon pea, co-inoculation of *Trichoderma harzianum*and AMF significantly improved plant growth and reduced *Fusarium udum* wilt severity [42]. The combination of these beneficial fungi can provide synergistic effects on disease control, particularly against *Fusarium* wilt. However, the effectiveness of this interaction depends on the selection of appropriate AMF species and *Trichoderma* strains. While most investigations indicate positive outcomes, some reports have noted potential negative impacts on plants. Overall, the use of AMF and *Trichoderma* as biostimulants aligns with modern trends in sustainable crop management and environmental conservation, offering a promising approach for enhancing crop productivity and plant health [2],[4].

1.2.5. Support for both plant growth promotion and immunity

the overall beneficial effects of *mycorrhiza* and *Trichoderma* that enhance plant growth and defense. Arbuscular mycorrhizal fungi (AMF) and *Trichoderma* species are beneficial microorganisms that enhance plant growth and defense mechanisms. These fungi establish mutualistic relationships with plants, triggering complex signaling events that promote growth and improve disease resistance. They modulate phytohormone signaling pathways, enhance nutrient uptake, and stimulate systemic acquired resistance through salicylic acid, jasmonic acid, and ethylene signaling [1],[43]. Metabolomic studies have shown that AMF and *Trichoderma* induce broad molecular reprogramming, particularly in the phenylpropanoid biosynthetic pathway, and accumulation of auxins, cytokinins, and jasmonates.[44]. These fungi act as elicitors of root system development, nutrient uptake, plant stress response, and production of secondary metabolites. While generally effective and safe for horticultural crops, some reports indicate potential negative impacts, necessitating further research [2].

The combination of *Trichoderma harzianum* and Arbuscular mycorrhizal fungi (AMF) has shown promising results in enhancing plant growth and disease resistance across various crops. Studies have demonstrated that co-inoculation of *Trichoderma harzianum*and AMF significantly improved growth parameters. These beneficial effects were observed in shoot height, dry weight and root development in tomato seedlings [11], [9]. pigeon pea [42]. Recent study was found that single inoculations were as effective as combined treatments in promoting maize growth under optimal nutrient conditions. The study also noted that these bio-inoculants can modulate native soil and rhizosphere microbial communities, which may influence their efficacy under various environmental conditions. [10]. In tea cuttings, tomato seedlings, and napier grass, *Trichoderma harzianum*and AMF enhanced growth, with *Trichoderma harzianum*potentially solubilizing minerals and producing growth hormones [11].

1.2.6. Support the soil healthy and structure

The interaction between arbuscular mycorrhizal fungi (AMF) and Trichoderma spp. can significantly contribute to soil health and plant growth in horticultural crops [2]. AMF play a crucial role in carbon flux, nutrient cycling, and soil aggregation, while their hyphosphere microbiome enhances nutrient cycling and carbon sequestration [45]. Combining biochar with AMF shows promise for improving soil health and crop productivity, although the mechanisms of their interaction require further research [46]. Sustainable agricultural practices, such as organic farming and conservation tillage, can enhance soil health by increasing microbial diversity and activity. AMF along with other beneficial soil organisms, improve water use efficiency, nutrient availability, and plant resistance to environmental stresses [47]. The interaction between arbuscular mycorrhizal fungi (AMF) and *Trichoderma* species can enhance plant growth and improve soil structure, particularly under stress conditions. Dual inoculation of AMF and *Trichoderma* has been shown to increase plant biomass, nutrient uptake, and stress tolerance in various crops [2],[48]. This synergistic effect is especially beneficial in saline soils, where the combination can improve the K+/Na+ ratio and modulate root metabolome [48]. AMF colonization enhances soil structure by increasing the production of glomalin-related soil proteins and improving water-stable aggregate stability [49]. However, the effectiveness of these interactions depends on the specific AMF species and Trichoderma strain used. While AMF and *Trichoderma* generally work synergistically, some studies have reported negative effects on plant growth and colonization levels, highlighting the need for careful selection of compatible species [4],[2].

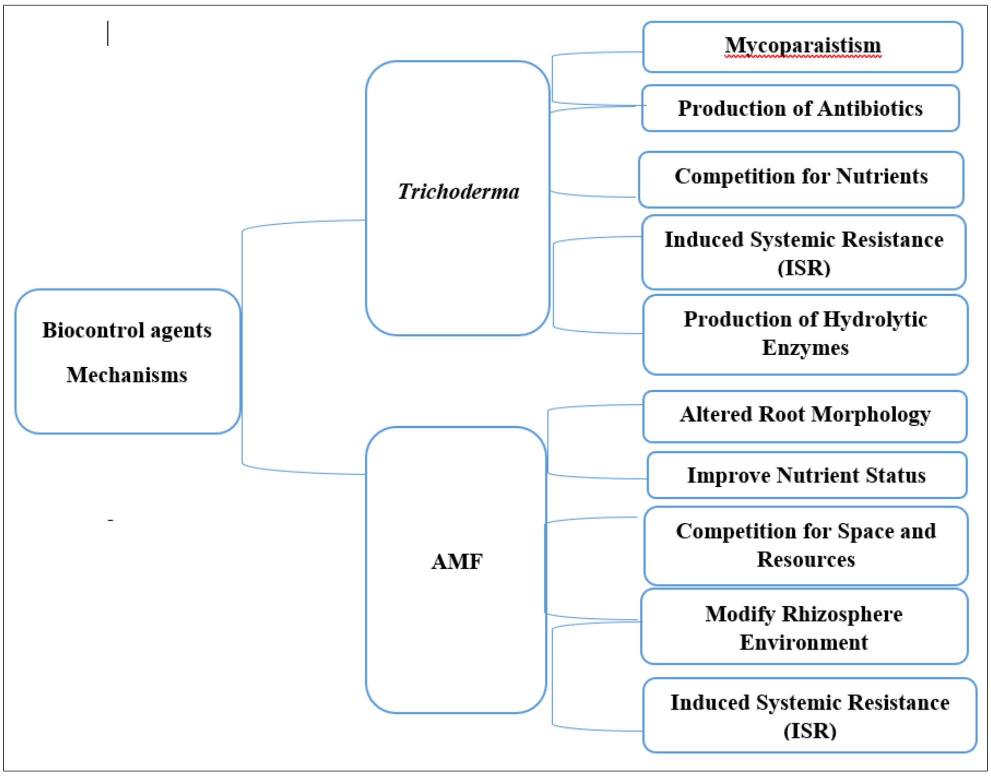

Figure 1 Schematic diagram shows the biocontrol agents mechanisms that using by *Trichoderma* and Arbuscular mycorrhiza fungi**.**

1.2.7. Synergistic Effects of Co-Inoculation

Co-inoculation of Arbuscular mycorrhizal fungi (AMF) and beneficial fungi like *Trichoderma* species has shown synergistic effects on plant growth, nutrient uptake, and disease resistance. Studies have demonstrated improved growth parameters, increased biomass production, and enhanced nutrient acquisition, particularly phosphorus and zinc, in various crops. This combination also significantly reduces the severity of fungal diseases, such as *Fusarium* wilt in pigeon pea and common bean. Furthermore, co-inoculation has been found to stimulate the production of health-promoting compounds, including phenolics and anthocyanins, in crops like black rice. The synergistic effects of AMF and beneficial fungi offer a promising, eco-friendly approach to sustainable agriculture, potentially reducing the need for chemical fertilizers and pesticides while improving crop yield and quality. [42],[50]-[52].

The co-inoculation of arbuscular mycorrhizal fungi (AMF) and *Trichoderma* species has shown synergistic effects on plant growth and disease resistance. Combined inoculation enhances nutrient uptake, particularly phosphorus and nitrogen, leading to improved plant development. This symbiotic relationship also increases plant resistance to pathogens, such as Fusarium wilt in melon and pigeon pea. The effectiveness of co-inoculation depends on the specific AMF and Trichoderma species used, with some combinations providing better results than others [4], [42], [53].

1.2.8. Applications in Agriculture.

Many of studies indicated to the synergistic effects of *Trichoderma harzianum*and AMF can enhance crop performance across different plant families. However, the specific impacts on native soil microbiota and the mechanisms underlying these improvements may vary depending on the crop and environmental conditions. The combined application of *Trichoderma harzianum* and arbuscular mycorrhizal fungi (AMF) has shown promising results in improving crop productivity across various plant species. In Brassicaceae plants, typically non-hosts for AMF, this combination enhanced root colonization and plant productivity [8]. Similarly, in maize, both single and combined inoculations of *Trichoderma harzianum*and AMF increased shoot dry weight [10]. For industrial hemp, co-inoculation resulted in increased root mass density, bud length, and CBD yield [54]. In field-grown tomatoes, nursery inoculation with *Trichoderma harzianum*and AMF improved plant growth, early yield, and fruit quality [20]. These microbial associations offer promising strategies for maintaining crop productivity under stressed environmental conditions. *Trichoderma spp*. and arbuscular mycorrhizal fungi (AMF) have shown promising results in enhancing plant tolerance to abiotic stresses like drought and salinity. *Trichoderma* can induce systemic resistance, improve root growth, and enhance nutrient uptake, thereby increasing plant tolerance to various stresses [3]. AMF colonization improves plant water status, root hydraulic conductivity, and nutrient acquisition under drought conditions. AMF also upregulate genes involved in K+/Na+ homeostasis and chloroplast function, contributing to salt tolerance [55]. Both *Trichoderma* and AMF help alleviate salt stress by enhancing antioxidant defense systems and osmotic adjustment [56], [55] . The mechanisms of stress mitigation by AMF include improved plant nutrition, accumulation of protective compounds, enhanced oxidative stress tolerance, and modification of plant physiology [57].

Co-inoculation of *Trichoderma* species and arbuscular mycorrhizal fungi (AMF) has shown promising results in controlling soil-borne pathogens and promoting plant growth in agricultural applications. Studies on pigeon pea demonstrated that combined inoculation of *Trichoderma harzianum*and AMF significantly reduced Fusarium wilt severity and enhanced plant growth [42]. Similar effects were observed in groundnut, where co-inoculation of *Glomus fasciculatum* and *Trichoderma viride* effectively controlled *Macrophomina phaseolina,* lowering disease incidence and severity while increasing biochemical and antioxidant activities [58]. The synergistic effects of these beneficial microorganisms are attributed to direct mechanisms like antibiosis and competition, as well as indirect effects such as plant growth promotion and induced resistance [59]. However, the effectiveness of co-inoculation can vary depending on the specific *Trichoderma* isolates used. Pre-treatment of soil with these biocontrol agents is recommended for optimal disease protection and growth enhancement [42].

1.2.9. Challenges and Considerations

Research on co-inoculation of arbuscular mycorrhizal fungi (AMF) and *Trichoderma* species has shown mixed results in plant growth promotion and disease control. While combined inoculation can enhance plant performance and suppress pathogens synergistically [4]. the effects may vary depending on specific strain combinations and environmental conditions [10]. Compatibility between AMF and *Trichoderma* is crucial for successful co-inoculation, as interactions can affect colonization levels and efficacy. Careful selection of compatible AMF and *Trichoderma* strains is essential for optimal results in improving plant growth, stress tolerance, and disease resistance in horticultural applications [4],[2]. Hyphal anastomosis, the fusion of fungal hyphae, plays a vital role in AMF compatibility and nutrient exchange, but can be hindered by genetic differences between isolates [60]. Environmental factors significantly influence the efficacy of *Trichoderma* and arbuscular mycorrhizal fungi (AMF) co-inoculation. Temperature plays a crucial role, with most *Trichoderma* strains being mesophilic and showing optimal activity at moderate temperatures Soil pH is another critical factor, with *Trichoderma* tolerating a wide range (pH 2.0-13) but preferring acidic conditions. Moisture levels also affect their performance, as *Trichoderma* strains struggle in dry conditions [61][62]. These environmental parameters not only impact the survival of biocontrol agents but also their ability to maintain biocontrol capacity. The co-inoculation of *Trichoderma* and AMF can enhance root system development, nutrient uptake, and plant stress response, However, their effectiveness as biostimulants depends on various factors, including soil texture, water content, pH, and crop history [63] , [2]. Field application of *Trichoderma* and arbuscular mycorrhizal fungi (AMF) can significantly enhance crop performance, but optimization of inoculation strategies is crucial. Studies have shown that both single and combined inoculations can improve plant growth, yield, and quality in various crops [64], [10], [65]. However, the effectiveness of inoculation depends on several factors, including species compatibility, field carrying capacity, and priority effects. Successful establishment of these beneficial microorganisms in agricultural soils requires careful consideration of inoculum choice, plant selection, management practices, and timing of application [66]. While combined inoculations can modulate native soil microbiota [10]. they may not always result in significant improvements over single inoculations under optimal conditions. Nevertheless, the use of microbial consortia has shown promising results in enhancing crop nutrition, productivity, and soil fertility [64].

2. Conclusion

The both microorganisms of *Trichoderma* and AMF have many of mechanisms to control plant disease. each of one have mechanisms some of these the same like induced systemic resistance and competition for nutrients while other mechanisms are unique for one of them like *Trichoderma* have ability to mycoparasitism, production of antibiotics and hydrolytic enzymes while the AMF have ability to altered root morphology, improved nutrient status and modify rhizosphere environment fig (1). Thus, from this important point these mechanisms are working together synergistically to enhanced plant growth and controlling soil-borne diseases like fungi and nematodes when invasion roots of plant. Many of studies which is indicated in their results that when using combine *Trichoderma* with Arbuscular mycorrhiza fungi (AMF) its more active from using them individually to improved plant growth and control the plant diseases. These findings suggest that co-inoculation strategies can enhance crop productivity in challenging environments.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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