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Research Article

Plant growth and nutrient uptake enhancement in thai dragon plant inoculated with arbuscular mycorrhizal fungi and actinomycetes

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Abstract
Soil-borne diseases pose a major threat to chili pepper production. This study investigated the synergistic effects of the arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* and the actinomycete *Streptomyces indiansis* on the growth, nutrient uptake, and disease resistance of Thai Dragon pepper plants challenged with *Fusarium oxysporum*. A pot experiment was conducted with (8 treatments), including individual and combined inoculations. Results showed that dual inoculation significantly enhanced plant morphology and nutrient uptake (N, P, K) compared to the control. To further understand the chemical changes associated with these treatments, a GC-MS analysis was performed using a SHIMADZU QP5050A system with an injection volume of (8.000 µL).Chemical analysis revealed that plants inoculated with symbiotic mycorrhizal fungi exhibited a distinct metabolic signature. In the group not treated with symbiotic mycorrhizal fungi, difluorodimethylsilane (DFMS) was detected at a retention time of 2.425 minutes, with a similarity index of 86 and a basal peak at 81.05 m/z. In contrast, the mycorrhizal-treated group showed cyclobutane (1-methylethylenediamine) at a retention time of 2.267 minutes, with a similarity index of 73. Most notably, chloroxylenol was detected at a retention time of 29.983 minutes, with a basal peak at 121.30 m/z, a high similarity index of 81, and a total ion intensity of 550,000 counts.The combined treatment effectively reduced the severity of Fusarium wilt, likely due to the identified bioactive compounds and hydrolytic enzymes. These results demonstrate that the three-part interaction among the eight treatments supports a sustainable biological control strategy, as evidenced by significant improvements in plant biomass and the presence of prominent metabolic markers identified by GC-MS.

Keywords: Biocontrol, *Fusarium oxysporum, Glomus mosseae*, , Plant growth-promoting rhizobacteria (PGPR), *Streptomyces indiansis*

INTRODUCTION

Chili peppers (*Capsicum annuum* L.) are a vital economic crop, playing a significant role in the global vegetable market. However, the sustainability of chili pepper production is severely threatened by soil-borne fungal diseases (Saba *et al*., 2022). One of the most notorious of these diseases is Fusarium wilt, caused by the pathogenic fungus Fusarium oxysporum, which poses a major ecological threat. This fungus aggressively colonizes the plant's vascular system, causing severe yellowing, wilting, and necrosis, leading to total crop loss and substantial economic losses. (Ahmad *et al.,* 2023). Due to the growing environmental and health concerns associated with synthetic fungicides, current research has shifted towards sustainable biological control strategies using beneficial rhizosphere microorganisms (Ahammed *et al.,* 2023).

Arbuscular mycorrhizal fungi (AMF), particularly species within the phylum Glomeromycota, establish symbiotic associations with most terrestrial plants and play a crucial role in agroecosystem resilience. *Glomus mosseae* (also known as *Funneliformis mosseae*) is one of the most effective species documented for enhancing plant performance. Recent studies have highlighted its capacity to significantly improve phosphorus (P) and micronutrient uptake by expanding the absorptive surface area of the root system (Begum *et al.,* 2022). Beyond nutritional benefits, *G. mosseae* confers bioprotection against soil-borne pathogens by triggering induced systemic resistance (ISR) and competing for infection sites (Ahammed *et al.,* 2023).

AM fungi are commonly , known for their biocontrol abilities, particularly their antagonistic activity against diseases of the roots caused by fungi and nematodes (Ravnskov *et al.,* 2020). Biocontrol attributes of Mycorrhizal fungi have been noted in Thai Dragonr (Reyes-Tena A., *et al.,* 2017, 2022). Several mechanisms of used by AM fungi for biocontrol have been suggested, including induction of plant protection (Banuelos, *et al*., 2014), enhance resistance to stress(Begum *et al*., 2019; Begum, 2019) raised nutrient consumption Thygesen *et al*., (2004), Whipps, (2004) modified exudation of root (Dowarah, 2021) and rivalry from mycorrhiza-associated bacteria (Kamal, 2014 , Kamal, 2007).

Concurrently, the rhizosphere hosts diverse populations of Plant Growth-Stimulating Rhizobacteria (PGPR), with Actinomycetes gaining prominence as potent biocontrol agents. Specifically, the genus *Streptomyces* is renowned for its ability to produce a vast array of bioactive secondary metabolites. These include siderophores for iron chelation and extracellular lytic enzymes such as chitinases and beta-1,3-glucanases (Kawicha *et al.,* 2023). Since the cell wall of Fusarium oxysporum is primarily composed of chitin, these chitin-degrading enzymes play a pivotal role in breaking down the fungal hyphae and preventing their spread (Zhu *et al*., 2023).

Although the individual benefits of mycorrhizae (AMF) and Streptomyces indiansis are well-established, their synergistic ability to protect chili peppers from Fusarium wilt remains limited. The interaction between mycorrhizae and specific PGPR bacteria, often referred to as "mycorrhizal helper bacteria" (MHBs), can improve plant health and enhance fungal colonization compared to the individual inoculation of either (Baniyaghob *et al*., 2024). Therefore, this study aimed to evaluate the tripartite interaction between *Glomus mosseae* and *Streptomyces indiansis* in improving the growth, nutritional status, and disease resistance of Thai dragon pepper plants under fungal stress induced by Fusarium oxysporum.

MATERIALS AND METHODS

Soil and substrate preparation

The experiment was carried out using a soil mixture consisting of peat, sand and vermiculite in a ratio of 3:1:1. The soil was steam sterilized three times at a temperature of 121°C and a pressure of 15 pounds per square inch for 50 minutes on consecutive days to eliminate native microbes and ensure suitable conditions.The soil was then placed in plastic pots (20 cm in diameter), and plants were fertilized with a modified nutrient solution (minus phosphorus) to ensure P uptake was dependent on mycorrhizal efficiency.

Microbial preparation

Arbuscular Mycorrhizal Fungi (AMF): The AMF species used was *Glomus mosseae*. Spores were originally obtained from the rhizosphere of chilli plants in Serdang, Malaysia, and propagated for 3 months using the pot culture method with maize (Zea mays) as the host and zeolite as the carrier substrate.

Bacterial Inoculum: The bacterial strain used was *Streptomyces indiansis*. This strain was obtained from the authors' culture collection, having been previously isolated and molecularly identified based on 16S rRNA gene sequencing (GenBank Accession No. [KP825180], Jalaluldeen, (2016). The strain was maintained on Starch Casein Agar (SCA) slants at 4°C. For inoculation, a bacterial suspension was prepared adjusting the concentration to approximately 10^8 CFU/mL.

Pathogen inoculum: The pathogenic fungus, Fusarium oxysporum, was cultured on potato and dextrose agar (PDA) plates and incubated at 25 ± 2 °C for 5–7 days until sporulation was complete. To prepare the inoculum, the plates were immersed in sterile distilled water, and the spores were gently scraped off. The suspension was filtered, the spore concentration was determined using a blood cell counter, and standardized to 1 × 10^6 spores/ml.

Experimental design and treatments

The experiment was designed using a completely randomized design (CRD) with a full factorial arrangement involving three factors: AMF (Glomus mosseae), pathogens (Fusarium oxysporum). and bacteria (Streptomyces indiansis), and this resulted in a total of eight treatments (n=3):

T_1: *Glomus mosseae* only

T_2: *Streptomyces indiansis* only

T_3: *Fusarium oxysporum* only (Pathogen control)

T_4: *G. mosseae + S. indiansis*

T_5: *S. indiansis + F. oxysporum*

T_6: *G. mosseae + F. oxysporum*.

T7: *G. mosseae + S. indiansis + F. oxysporum*
T8: Control (Uninoculated healthy plants)

Inoculation and plant management

Seedlings of Thai Dragon chili were transplanted into the prepared pots. AMF inoculum (10 g/pot) was placed below the seedling roots at transplanting. Bacterial suspension (10 mL/pot) was applied as a soil drench. For pathogen-challenged treatments, the *F. oxysporum* spore suspension (10 mL/pot) was applied to the soil around the root zone two weeks after transplanting to simulate natural infection pressure.

Shoot growth

From week 6 to week 10, while they were in growth stage, the plant height was measured five times. The plant was harvested under cold weather to minimize water lose. plants were then placed in an oven at 70 °C for 24 hours to determine their dry mass.

Root morphology

Measurements were taken of the root tips, total root length, surface area, and volume at the conclusion of the experiment using a root scanning analysis device (Tahat *et al.*, 2008).

Chlorophyll content

After adding 20 ml of an 80% aqueous acetone, five discs of fresh chili pepper leaves were extracted and left to sit for three to six days. The absorbency of chlorophyll (a) and chlorophyll (b) was measured using Spectrophotometer at 647nm and 664nm respectively.
Depending on these followed equations Chlorophyll (a) and (b) contents as well as the total amount of leaf chlorophyll content were examined.
ch.(a) =13.19 A664-2.57A647 mg/gm of fresh weight
ch.(b) =22.10 A647-5.26 A664 mg/gm of fresh weight

$$\text{Total chlorophyll} = \frac{3.5\,(\text{ch.a}+\text{ch.b})}{5} \times 100$$

A =wave length, 3.5=total of ch. Extract in vial, 5=area of leaves for ch. Extract following standard methods (APHA, 1992).

Shoot and root nutrient analysis

Wet washing method as elaborated by (Sharifuddin, H.A., 1984), was followed. Dry plant shoots and roots (0.25g) were put in a beaker. 5 mL of 100% H2SO4 was added to each sample. This was followed by heating the samples on a hot plate at 450 °C for 15 min. 10 millilitres of concentrated H2O2 were incorporated to each sample and heated for 7 min. At 450 oC. The quantities of macronutrients K, P, and N, and the micronutrients Mg, Fe, Zn, and Ca were quantified using an auto-analyzer system in the Department of Land Management, UPM.

Data collection

Plants were harvested 10 weeks after transplanting. Growth parameters including plant height, shoot and root dry weight (dried at 70°C for 72 h) were recorded. Root morphology (root length, surface area, and volume) was analyzed using a root scanner. Chlorophyll content (a, b, and total) was measured spectrophotometrically. Macro- and micronutrient content in shoots and roots (N, P, K, Ca, Mg, Fe, Zn) was analyzed using standard wet-digestion methods followed by auto-analyzer analysis.

Statistical analysis

Data were analyzed using SAS software (Version 9.3). Prior to statistical analysis, data normality was verified using the Shapiro-Wilk test, and homogeneity of variance was checked using Levene's test. Data were then subjected to one-way Analysis of Variance (ANOVA). Means were compared using Tukey's Honestly Significant Difference (HSD) test at a significance level of P\le 0.05. All values are reported as mean ± Standard Deviation (SD).

RESULTS AND DISCUSSION

Arbuscular Mycorrhizal Fungi (AMF) colonization

The observation of different structures, such as hyphae, arbuscules, and vesicles, in plant root tissues indicates colonisation of the root by AMF (Sahu and Sindhu, 2011). Despite the fact that the host plant's root tissue possesses only one AMF organ, a mutually beneficial relationship has formed. AMF-colonized roots are shown in (Fig. 1).
The Fig. also demonstrates uninfected and AMF-infected plant root tissue, including hyphae and vesicle spores. Hyphae have fine-threads shape; spores are circle to oval with distinct brownish color. AMF interior hyphae bulge to form vesicles, which might be noticed within or outside the parenchyma cortical layer. The range of colonization of plant root by AMF indicates their ability to establish colonies on the host. This elevated colonization range shows how dependent and necessary plants are for AMF to exist,
Influence of Microbial Inoculants on Plant Growth and Biomass
The experimental results demonstrated that co-inoculation with *Glomus mosseae* and *Streptomyces indiansis* significantly enhanced the growth of Thai Dragon chili plants compared to the uninoculated control (P \le 0.05). Double inoculation (T_4: mycorrhizae + bacteria) resulted in the highest vegetative growth rates, with a significant increase in plant height and biomass. This effect is attributed to the mycorrhizal helper bacteria (MHB) hypothesis, where Streptomyces species facilitate mycorrhizal spore germination and hyphae expansion (Banyagoup *et al*., 2023).

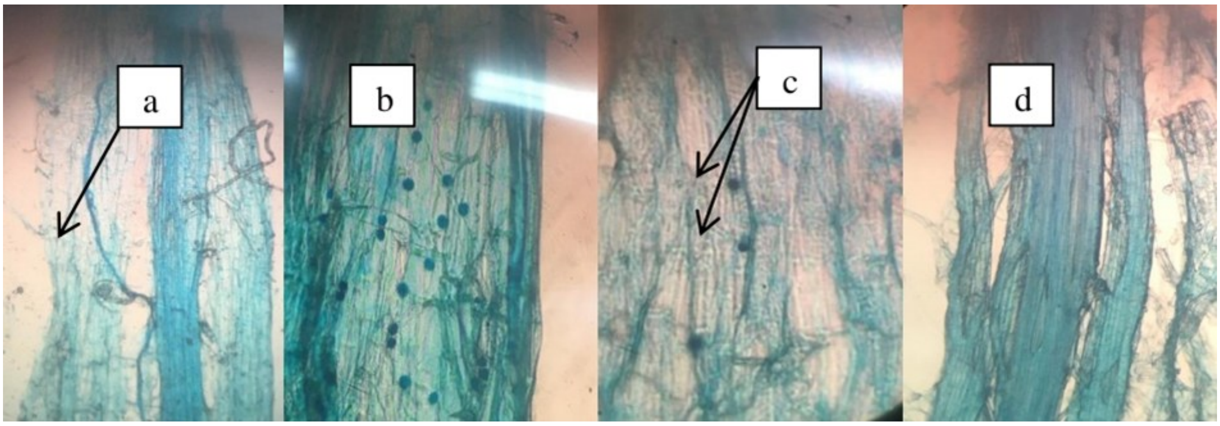

Fig. 1. *AMF-infected roots (a) with hyphae, (b) with vesicles, (c) with spores, and (d) infection-free*

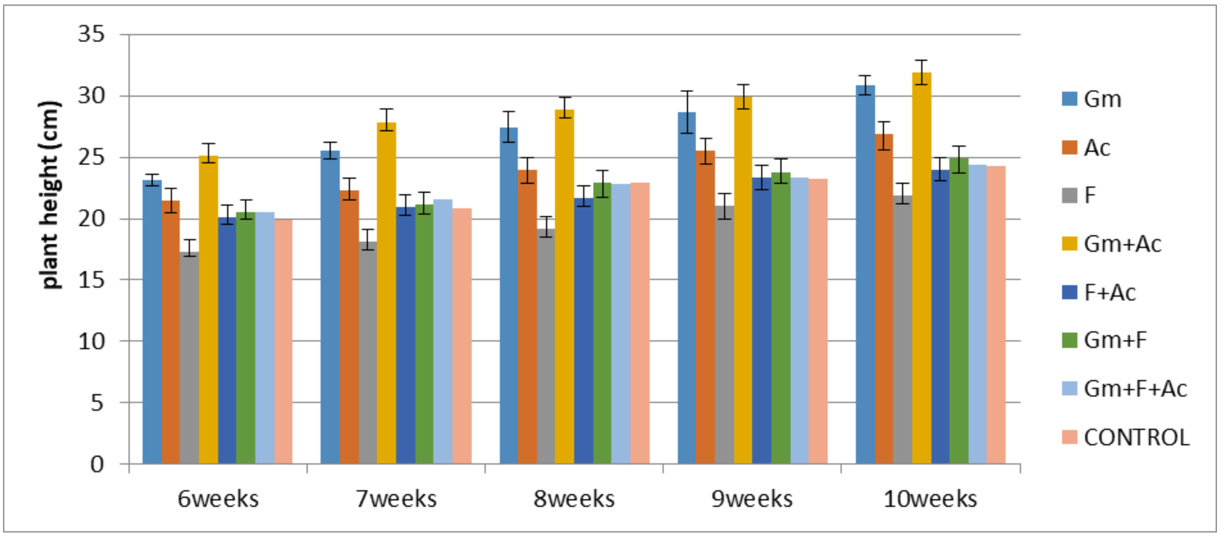

Fig. 2. *Effect of Glomus mosseae (Gm), Actinomycetes (Ac), and Fusarium oxysporum (F).*

In contrast, plants infected with Fusarium oxysporum alone (T_3) exhibited the lowest growth rates due to the systemic nature of the wilt. However, the triple interaction (T_7: *Glomus mosseae* + Bacteria + Pathogen) showed remarkable recovery, maintaining biomass levels significantly higher than those in the pathogen control (T_3) (Fig. 2). inoculation on plant height of Thai Dragon chili from the 7th to the 10th week of growth. Vertical bars represent the Standard Deviation (SD) (n=3), and individual data points are overlaid to show the distribution.

This suggests that the presence of beneficial microbes compensates for the energy drain caused by the pathogen, (Vimal *et al.,* 2022).

Root architecture and synergistic morphological modulation

Thai plants inoculated with *Glomus mosseae* and Actinomyces showed excellent root development in contrast to those inoculated with the other treatment and the control (Fig. 3).

Glomus mosseae combined with Actinomycetes treated plants had a considerably higher number of root tips comparision with the pathogen and control. Compared with the control and others, the plant treated with Actinomycetes had much longer roots, greater volume, and a larger surface area. The plants that received the AMF plus Actinobacteria treatment had the best overall girth, followed by those that received the AMF inoculation alone. The leaf surface area of plantinitially inoculated with AMF and Actinobacteria was the largest, showing a progressive increase over time. AMF established strong interactions with these plants leading to abundant growth. spore numbers in the soil. Plants inoculated with *Glomus mosseae +F.oxysporum* significantly reduced the effect of the disease caused by F.oxysporium based on the numerical of root peaks, extent, outside, and volume (Fig. 4).

Root scanning analysis revealed a profound shift in root system architecture. Dual inoculation (T_4) and the tripartite system (T_7) significantly increased total root length, surface area, and root volume.

In Fig. 4 the significant improvement in chili growth and flowering under the dual inoculation of Gm + Ac suggests a high degree of compatibility between *G. mosseae* and *S. indiansis*. This enhancement is likely due to the root system's increased efficiency in absorbing immobile nutrients. According to Kawicha *et al.,*

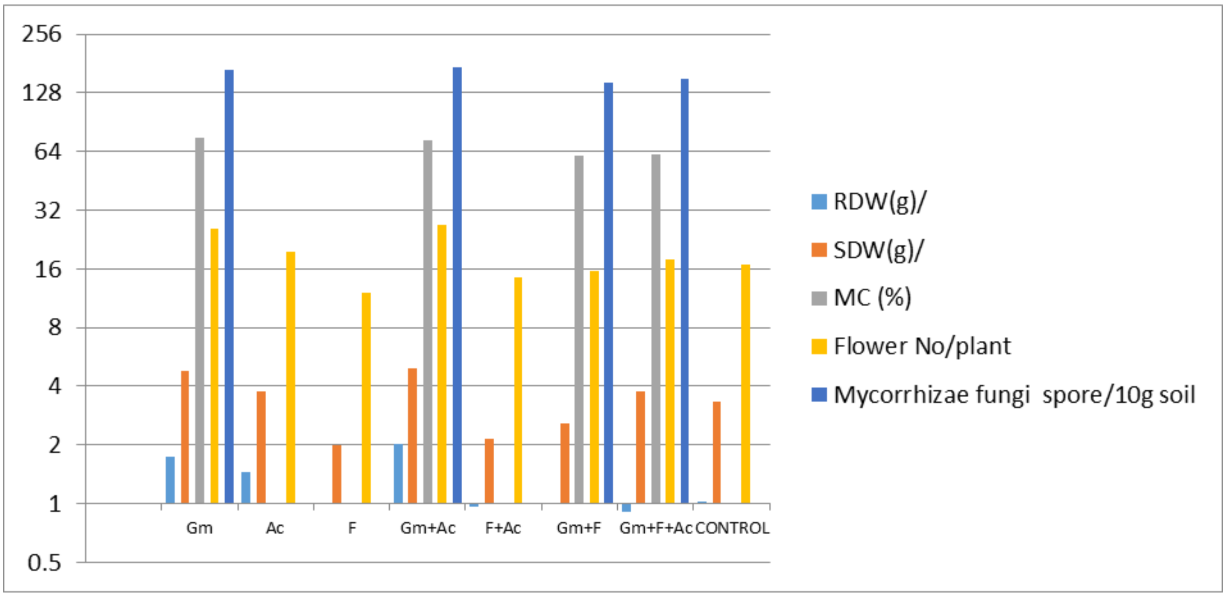

Fig. 3. *Response of root dry weight (RDW), shoot dry weight (SDW), mycorrhizal colonization (MC), and flower number to (Gm) and (Ac) treatments after 10 weeks of chili growth. Mean values followed by the same letter are not significantly different according to Tukey's Honestly Significant Difference (HSD) test ($P < 0.05$). Error bars represent the Standard Deviation (SD).*

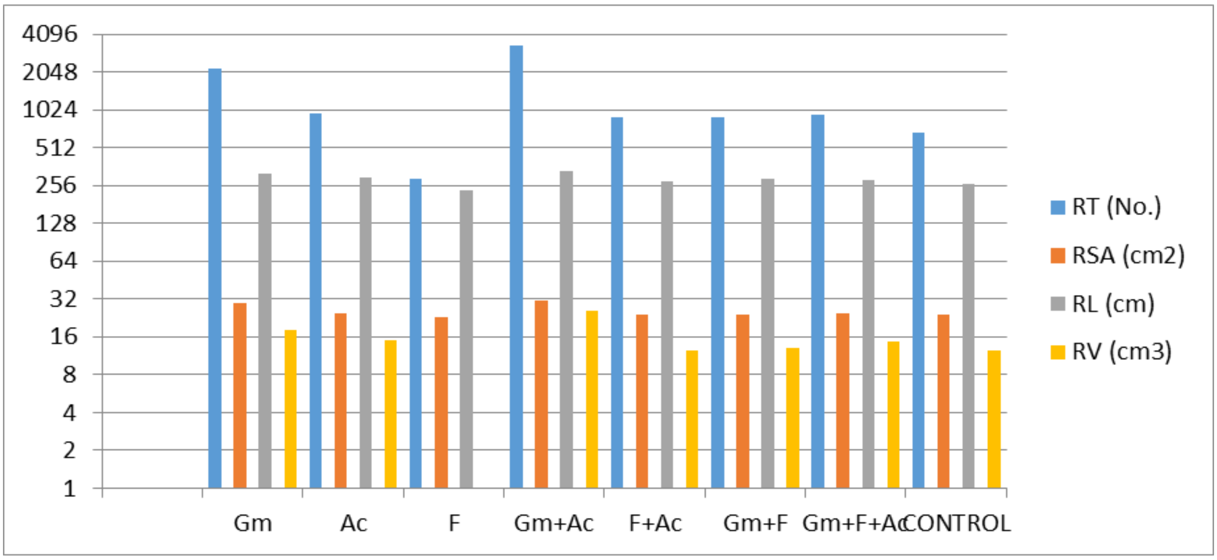

Fig. 4. *Effect of (Gm) and (Ac) on root architectural characteristics of Thai Dragon chili: root tips (RT), root volume (RV), root surface area (RSA), and root length (RL) after 10 weeks of growth. Mean values followed by the same letter are not significantly different according to Tukey's HSD test ($P < 0.05$). Values are presented as mean ± SD (n=3).*

(2023), *Streptomyces* species isolated from the rhizosphere possess the ability to solubilize phosphates and produce indole-acetic acid (IAA), which directly stimulates root elongation and dry matter accumulation. This supports present findings, which showed that the highest root dry weight (2.02 g) was observed in the dual treatment.

Furthermore, the increase in flowering (27.00 flowers/plant) in the Gm + Ac treatment highlights the role of these microbes in modulating plant physiological pathways. Ahammed *et al.,* (2023) noted that arbuscular mycorrhizal fungi can alter the source-sink relationship in plants, favoring the transition to the reproductive phase through improved carbon and mineral allocation. Beneficial bacteria play a pivotal role in this context; Olanriwaju and Babalula (2019) indicated their effectiveness as biostimulants that enhance plant vitality and improve flowering rates. On the preventative side, the triple treatment (Gm + F + Ac) has proven highly effective in combating diseases. resulted in a higher shoot dry weight (3.78 g) than the pathogen only treatment. This biological protection is attributed to what is known as the 'inducible systemic resistance' (ISR) mechanism; Nadeem and colleagues (2022) confirmed that the joint and cooperative action between mycorrhiza fungi and friendly bacteria effectively activates the plant's defense

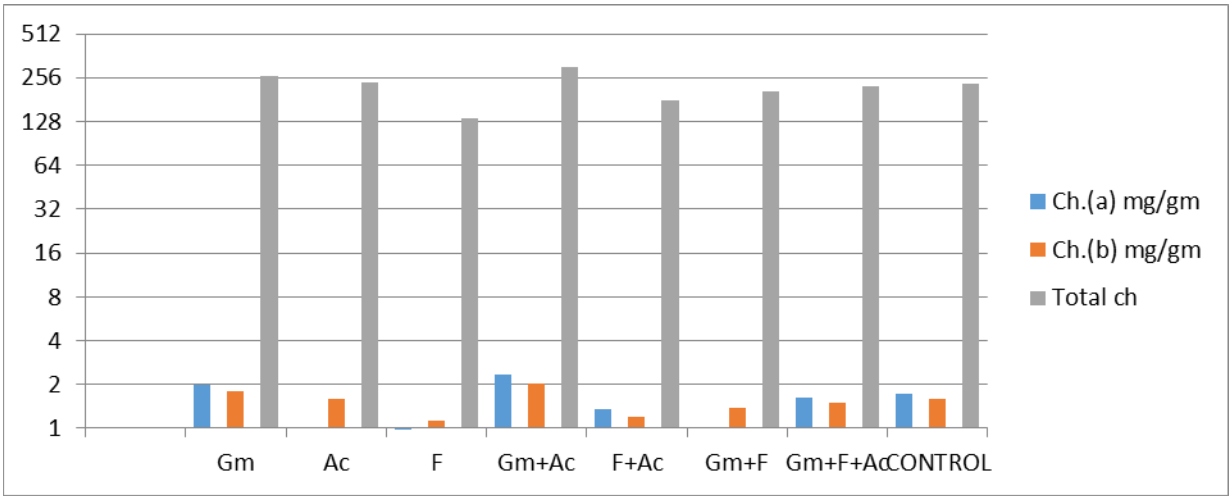

Fig. 5. *Effect of (Gm) and (Ac) on chlorophyll a (ch.a), chlorophyll b (ch.b), and total chlorophyll (Total ch.) content in Thai Dragon leaves after 10 weeks of plant growth. Mean values followed by the same letter are not significantly different according to Tukey's HSD test (P < 0.05). Error bars represent the Standard Deviation (SD).*

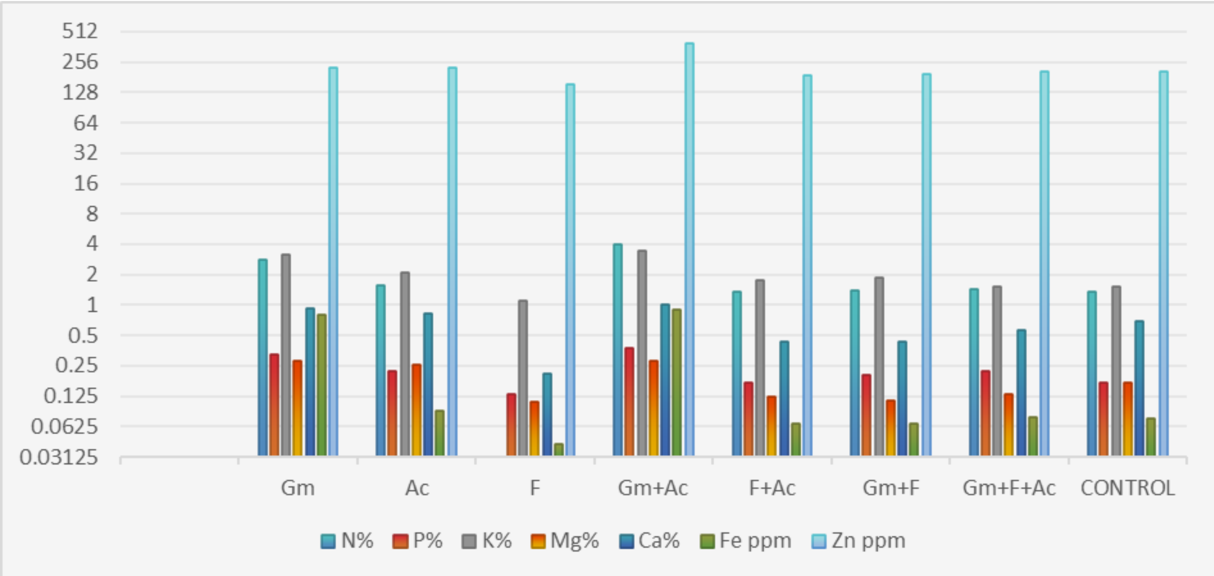

Fig. 6. *Effect of (Gm) and (Ac) on the macro- and micro-nutrient concentrations in chili shoots after 10 weeks of growth. Mean values within columns followed by the same letter are not significantly different according to Tukey's HSD test (P < 0.05). Values represent mean ± SD (n=3).*

system., thereby making it more resilient to Fusarium colonisation. Additionally, the maintenance of substantial mycorrhizal colonisation (62%) in the presence of the pathogen suggests that *S. indiansis.* This may contribute to the formation of a protective layer surrounding the fungal hyphae, ensuring the sustainability and stability of the symbiotic relationship even under stress conditions, as discussed by Kamal *et al*. (2014). This structural development is likely due to the production of the hormone indole-3-acetic acid (IAA) by S. indiansis; this compound stimulates lateral root development and root extension in the soil via symbiotic hyphae (Safeena *et al*., 2024). The importance of this robust root structure lies in enhancing the plant's immunity against the negative consequences of Fusarium fungal spread in the root zone.

Chlorophyll content and photosynthetic resilience

Colonization of the roots by the fungus Fusarium led to a sharp decrease in chlorophyll concentrations, causing yellowing and impaired photosynthesis. However, the addition of G. mosseae and *S. indiansis* provided protection, enabling the plant to retain 85% of its chlorophyll content. This phenomenon is explained by the ability of these organisms to balance oxidative stress and ensure nutrient delivery to the leaves, thus maintaining stable carbon uptake under pathogenic stresses (Ahmed *et al*., 2023).

Nutrient uptake dynamics (N, P, K, Fe, and Zn)

The results of elemental content estimation demonstrated that double inoculation led to a significant increase in the extraction of essential macro and micro-

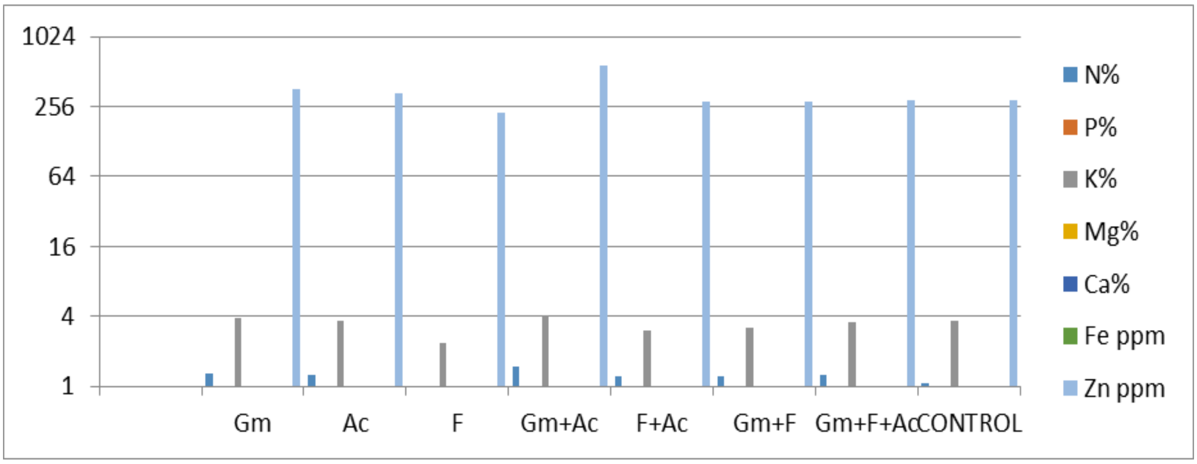

Fig. 7. *Effect of (Gm) and (Ac) on the macro- and micro-nutrient concentrations in Thai Dragon chili roots after 10 weeks of growth. Mean values within columns followed by the same letter are not significantly different according to Tukey's HSD test (P < 0.05). Values represent mean ± SD (n=3)*

nutrients (Fig. 6).

Phosphorus (P): Clear growth spurts were recorded in all treatments enhanced with G. mosseae (T_1, T_4, T_6, T_7), confirming the efficiency of mycorrhizae in facilitating phosphorus uptake and transport through their hyphae extending into the soil (Begum *et al*., 2022).

Micronutrients (Fe, Zn): The highest accumulation of iron and zinc was found in treatments involving *S. indiansis*. This is likely due to the secretion of siderophores by the actinomycete, which chelate poorly soluble ions in the soil and make them available for plant uptake (Fig,7) (Zhu *et al.,* 2023).

The study observed that the significant increase in nutrient uptake (N, P, and K) and microelements (Fe and Zn) observed in the Gm + Ac treatment (Fig. 5, 6) aligns with the recent findings of Safeena *et al.,* (2024), who demonstrated that *Streptomyces* strains are potent siderophore producers that enhance iron and zinc mobilization in the rhizosphere. Furthermore, the synergistic interaction between AMF (*G. mosseae*) and *Streptomyces* in our study confirms the "helper effect" theory proposed by Baniyaghob *et al.,* (2023), in which beneficial bacteria promote mycorrhizal colonisation, leading to improved plant biomass and nutrient status even under pathogen pressure. The reduction in nutrient content in plants infected with *Fusarium* alone (F treatment) is consistent with the pathogen's mechanism of colonizing vascular tissues, as described by Ahmad *et al.,* (2023).

Biocontrol mechanisms and disease suppression

The current results highlight that the triple treatment (T_7) was the most effective in reducing the severity of *Fusarium* wilt infection. Unlike other oomycetes, Fusarium oxysporum has a cell wall composed primarily of chitin; hence, the inhibitory effect of S. indiansis stems from the secretion of chitinase and beta-1,3-glucanase enzymes, which enzymatically degrade the fungal hyphae (Olanrewajo and Babalola, 2019). In parallel, *G. mosseae* plays a protective role by inducing systemic resistance (ISR), thereby activating the plant's defense system. The AMF leads to earlier and stronger activation of defence-related enzymes, such as polyphenol oxidase (PPO), peroxidase (POX) and thereby This interaction results in a biochemical shield that repels pathogen attacks, as explained by Nadeem *et al*. (2022). The exceptional performance of the triple-layered combination is attributed to the integration of two mechanisms: direct bacterial antagonism and innate stimulatory support, a process known as the 'dual defense' approach.

Study limitations and ecological context

"In this research, a steam-treated soil model (sand-Lovisole mixture) was used to ensure a sterile growing environment suitable for the study conditions. Despite the precise isolation of microbial interactions in this experiment, the absence of natural microbial competition remains a significant factor; introduced strains face dominance challenges with native microbes in open fields (Poudel *et al*., 2021). Therefore, the *in vitro* confirmed efficacy of present strains requires field validation in more complex culture environments to verify their consistent performance under non-sterile conditions.

Conclusion

In conclusion, The current study revealed the complementary capabilities of double insemination. the fungus *G. mosseae* with the actinomycete *S. indiansis* to promote the growth and health of Thai Dragon chilli plants. The combined biological system of triple treatment (T_7) proved more effective than single-drug applications in promoting plant growth and strengthening the root system, as well as improving the uptake of macro- and micronutrients. This biological assembly played a crucial role in suppressing *F. oxysporum*, leveraging the synergistic ability of the mycorrhizal fungi to provide food and the ability of *S. indiansis* to secrete enzymes that degrade the pathogen's cell walls. These findings suggest the potential of this three-way interaction as an environmentally friendly option for managing soilborne diseases in chili pepper crops, provided that the sustainability of this performance is confirmed through extensive field studies that take into account the complexities of the agricultural environment.

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Conflict of interest
The authors declare that they have no conflict of interest.

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