Research Article

Enhancing Induced Resistance In Tomato Plants Against Root Wilt Disease Using The Mycorrhizal Fungus Glomus Mosseae And Its Impact On Secondary Phenolic Compound Accumulation

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Abstract:
Root wilt is a devastating disease affecting tomato (Solanum lycopersicum L.) yields globally and is caused by soil-borne fungal pathogens. PurposeThis study was initiated to test the effect of the arbuscular- mycorrhizal- fungus (AMF), Glomus mosseae (GM), on the expression of induced systemic resistance against the wilt pathogen of areca palm, Fusarium brachygibbosum (Fb). A greenhouse experiment was performed under four treatments: Control, GM-only (T1), Fb-only (T2) and a mixture of GM+Fb (T3). The findings showed that GM inoculation resulted in considerably higher growth and physiological attributes. At the 10th week, GM alone resulted in the tallest plants (94.75 cm) and flowering of plants (mean = 6.5) only in the treated group. Inoculation with Fb elicited drastic growth retardation and decreased total chlorophyll to 354.82, however, dual inoculation (GM+Fb) dramatically recovered plant height (77.75 cm) and chlorophyll content (454.65). THE TPC measurement showed a remarkably powerful "priming" effect in GM + Fb treatment which was the largest among the groups (226.42 mg/g), the group with the highest TPC only containing Fb (179,62 mg/g). In addition, the activities of defence-related enzymes, Peroxidase (POD) and Polyphenol Oxidase (PPO), peaked in GM+Fb group (0.11 and 0.62 respectively), suggesting a strong enhancement of the antioxidant protection of plants. ConclusionsCollectively, these data support the conclusion that G. mosseae protects pine seedlings from F. brachygibbosum by a morphological and biochemical synergetic mechanism of resistance. From an applied perspective, this relationship embodies an environmentally sound and sustainable approach to control root wilt diseases within integrated pest management programs. Giuseppe G. Casaluce, 2015

Keywords: Induced Systemic Resistance (ISR); Solanum lycopersicumL; Quercetin; Biocontrol; Arbuscular Mycorrhizal Fungi (AMF); Glomus mosseae; Plant Defense; Secondary Metabolites,Disciplinary E, Journal of Applications and Social Sciences, E-colour Press, Research K Press, 543 45th Street, New YorkWrite a commentYour EmailYou may use these HTML tags and attributes:.

1-Introduction:

Tomato (Solanum lycopersicum L.) is one of the most prominent vegetable crops in the world and plays a critical role in human nutrition as well as the world agricultural economy. As a source of bioactive compounds (like lycopene, vitamins and other essential antioxidants) it is a basic component of the Mediterranean diet and an important value commodity for processing industries and fresh markets. Nevertheless, an extensive range of soil-borne phytopathogens subject sustainable tomato production to continuous and severe threats. Root wilt diseases are among these, specifically caused by fungal pathogens, constituting an important constraint. These pathogens enter the vascular system resulting in the clogging of vessels and non functioning, blocked or dead vessels cannot deliver water to the plant resulting in irreversible wilting the death of the plant. Such infections are capable of causing reproductive yield loss exceeding 80% in favorable, moist conditions (Tariq et al., 2023). Over the past centuries, the control of fungal wilt has extensively relied on the use of synthetic chemical fungicides. Though these conventional methods gave short-term relief, their continued and indiscriminate application has caused serious environmental and public health risks over the years. Chemical residues that persist in the food chain, the indiscriminate contamination of groundwater, and the massive loss of many types of soil microbial biodiversity are major disadvantages, however. Moreover, the appearance of resistant fungus strains to fungicides made it unviable to use traditional treatments (He et al. 2021). Therefore, the transition towards green and organic biocontrol methods has emerged as a worldwide challenge in contemporary integrated pest management (IPM)..

Arbuscular Mycorrhizal Fungi (AMF) are found to be biological alternatives to improve the health and stress tolerance of the plants in this regard. Glomus mosseae is one of the AMF species that has the highest efficiency as a symbiont in the tomato root. This symbiotic relationship not only improves the nutrient uptake especially phosphorus, micronutrients, and tolerance to drought, but its benefits stretch much further. In contrast, G. mosseae exerts an indirect biocontrol function against soil-borne diseases by stimulating the plant's innate

immune system via a process that we term Induced Systemic Resistance (ISR). The mycorrhizal symbiosis also "primes" the host plant via ISR to mount a faster and stronger defense response when secondarily challenged by the root wilt pathogen (Fusarium brachygibbosum) (Dowarah et al., 2022).

Biochemically, this induced resistance mechanism is rooted in the dramatic reprogramming of the plant's secondary metabolism. In this hierarchy of defence mechanisms at the chemical level, secondary phenoliccompounds play a leading role. These metabolites were shown to exhibit strong antimicrobial (phytoalexin) activity which can directly suppress fungal spore germination and mycelial growth also disrupt the enzymatic activity of the invading pathogen (Yadav et al., 2021). In addition, the increase in total phenolics is often connected with the higher activity of defense-related enzymes like peroxidase (POD) and polyphenol oxidase (PPO) [24]. These enzymes also catalyze the oxidation of phenols into highly toxic quinones and promote the sequence of lignification. The plant builds a physical barrier that prevents the invasion and colonization of F. brachygibbosum from the infected roots after reinforcing the host cell walls with lignin (Gupta et al., 2022)..

While the benefits of AMF for promoting plant growth are well-documented, the quantitative dynamics of phenolic compound accumulation—and their partitioning between roots and leaves following an active infection by F. brachygibbosum—are poorly understood. However, a crucial knowledge gap is the relationship between the extent of mycorrhizal colonization, the intensity of the biochemical defense response, and the level of reduction in disease severity. These associations must be recognized in order to maximize the application of G. mosseae as a viable bio-immunizer in tomato cultivation.

Thus, in the present study, we evaluated the effect of Glomus mosseae on induced resistance against root wilt disease incited tomato plants. Specifically, the research aimed to:

1. The key connected objections of the examination were to assess the mycorrhizal colonization productivity and its biocontrol potential to reduce disease severity index (DSI).
2. Take in to account quantifying total secondary phenolic compounds accumulation in leaf and root tissues in response to pathogen inoculation.

Elucidate the correlation between these biochemical changes and the alleviation of wilt symptoms.

3. This study aims to achieve these objectives to gain a deeper insight into the role of mycorrhiza-mediated phenolic accumulation as a sustainable strategy to protect strategic crops against emerging soil-borne threats.

2-Materials and Methods

2.1. Plant Material and Growth Conditions Seeds of tomato (Solanum lycopersicum L.) were surface-disinfected using 2% sodium hypochlorite for 3 minutes, followed by multiple rinses with sterile distilled water. The experiment was conducted in a greenhouse under controlled conditions (25/18°C day/night temperature, 16 h photoperiod, and 65% relative humidity).

2.2. Microbial Inocula Arbuscular Mycorrhizal Fungus (AMF): The inoculum of shrubby mycorrhizal fungi (AMF) was obtained from healthy and mature spores selected under a binocular microscope from the Institute of Plant Pathology Research, Agricultural Research Center, China. The mycorrhizae were added at planting directly by placing them near the root zone, and then the plants were lightly watered. The plants were left for two months to allow the roots to be colonized by the mycorrhizae before adding the pathogenic fungus.

2.3. Pathogen Isolation and Inoculum Preparation

Among the pathogens tested, the root wilt pathogen, Fusarium brachygibbosum, was first isolated from soil samples of the rhizosphere zone of tomatoes infected with the root wilt, in October 2023. The soil dilution plate method was employed for the isolation on a selective medium. Molecular characterization of the fungal isolate was performed to confirm the genus, where genomic DNA was isolated and Polymerase Chain Reaction (PCR) was performed with internal transcribed spacer (ITS) universal primers.

Inoculation on Potato Dextrose Agar (PDA) was performed and the pathogen was incubated for 7 days at 25 °C post molecular confirmation. Mycelium was harvested by flooding the culture plates with sterile distilled water and scraping the mycelium to prepare a conidial suspension. This suspension was then filtered through double-layered cheesecloth, and concentrated to a final concentration of (10^6) conidia/mL based on a hemocytometer.

2.4. Experimental Design and Treatments: During the whole investigation, a completely randomized design (CRD) was managed including four treatments: [4] replicates per treatment:

C: Control, plants grown in sterile soil without inoculation.

Mycorrhiza (M): Plants inoculated with G. mosseae only at sowing time

Pathogen (P): Plants challenged with wilt pathogen only (20 days after growth).

Combined (M + P): Plant infected with G. mosseae followed by pathogen challenge.

2.5. Disease Properties The disease severity index (DSI) was measured [30 days] after pathogen inoculation with a visual scale from 0 to 4 (0 = healthy, 4 = completely wilted). The percentage of protection was calculated by comparing DSI in mycorrhizal plants with that in non-mycorrhizal infected plants.
2.6. 2.1 Root and leaf samples were gathered at the conclusion of the experiment for quantification of Total Phenolic Compounds. Total phenolic content was measured using the Folin-Ciocalteu method. In short, 0.5 g of fresh tissue was extracted in the methanol 80% 1:9. Sodium carbonate and Folin-Ciocalteu reagent were added to the extract. Absorbance at 765 nm was measured with a spectrophotometer. The results are expressed as mg of GAE/g of fresh weight.
2.7. Mycorrhizal Colonization Assessment Roots were cleared with 10% KOH and stained with 0.05% Trypan Blue. To confirm the symbiosis formed by G. mosseae, the percentage of mycorrhizal colonization was calculated by using the gridline intercept method (Giovannetti and Mosse, 1980).
2.8. Statistical Analysis Data were analyzed by one-way ANOVA with [SAS]. Mean separations were performed using DM RT with P \leq 0.05..

Results And Discussion:

3.1. Isolation and Molecular Identification

The fungal isolate obtained from the tomato rhizosphere soil exhibited characteristic cottony white to pinkish mycelial growth on PDA. Microscopic examination revealed the presence of typical macroconidia and microconidia consistent with Fusarium spp.
The molecular identification via PCR amplification of the ITS region yielded a fragment of approximately 500 bp (Figure 1). Subsequent DNA sequencing and BLAST analysis confirmed the isolate as Fusarium brachygibbosum with a high identity percentage (e.g., 99-100%) with Accession number(PZ239910).

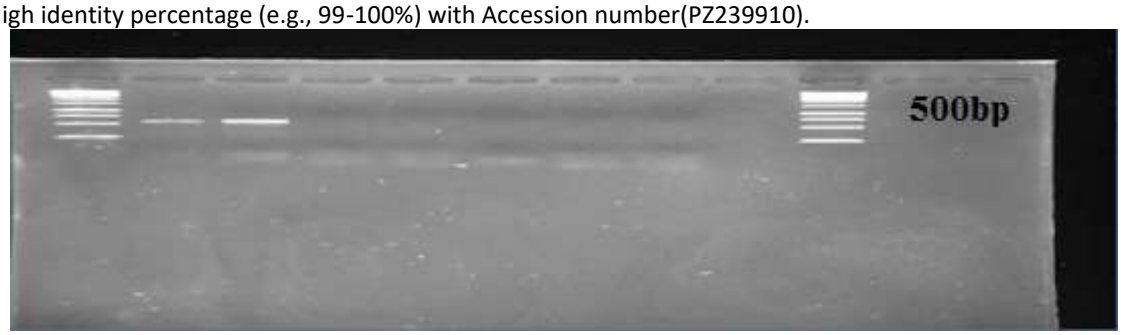


Figure 1. Typical ethidium bromide-stained gel electrophoresis pattern of DNA extracted from Fusarium isolates following a large-scale extraction protocol, molecular weight marker in the M pathway (DNA ladder mix).

3.2. Impact of AMF on Morphological Growth and Biomass Partitioning

In contrast, inoculation with Glomus mosseae effectively mitigated the detrimental effects of fungal stress on vegetative activity of tomato plants, which has been in accordance with the pathogenesis of Fusarium brachygibbosum. Significant differences among treatments in fresh weight, root height, shoot dry weight, and root dry weight were observed ($p \leq 0.05$) in (Figure 2). The tomato plants inoculated with G. mosseae alone showed the maximum values for all growth parameters measured with values of fresh weight (168.50 g), root height (68.00 cm), shoot dry weight (11.47 g), and root dry weight (22.82 g). Conversely, plants infected with F. brachygibbosum alone exhibited the lowest values reflecting a high extent of plant growth suppression from pathogen stress.
The decrement in growth parameters observed under the F. brachygibbosum pathogen infection may be related to the damage done on the root tissues, vascular discoloration and disturbance of physiological processes which caused impaired uptake of nutrients. Fusarium species induce oxidative stress, affording inhibition of water transport and occurance of photosynthetic activity, reducing the aboveground and belowground biomass (Juárez et al. Recent studies reveal these pathogenic Fusarium species block vascular development and produce toxins that significantly reduce tomato shoot and root development with similar reductions in growth under Fusarium infections..
On the other hand, inoculation with G. mosseae significantly promoted growth of tomato in comparison to non-inoculated plants and reduced the adverse effects of pathogen infection. The AM fungi enhance the tolerance of the plant against soil-borne pathogens through multiple mechanisms such as improving nutrient uptake,

stimulating antioxidant enzyme activity, strengthening the root cell walls, and inducing systemic resistance. The greater biomass accumulated in the GM treatment indicates that mycorrhizal colonization increased host physiological performance while controlling plant nutrient balance under stress conditions from fungi. This finding is also reported by Begum N. et al. They provide evidence that AMF inoculation leads to substantial increases in plant growth and stress tolerance through enhanced nutrient acquisition and defence response (2024)..

The combined treatment (GM + Fb) produced significantly higher growth values than plants infected with F. brachygibbosum alone, although values remained lower than those observed with GM alone. This finding indicates that G. mosseae partially reduced the pathogenic impact of F. brachygibbosum infection. The protective role of AMF against Fusarium diseases has been widely documented in tomato plants, where mycorrhizal colonization reduced disease severity and improved plant resistance under biotic stress conditions. Recent studies showed that AMF decrease pathogen colonization by competing for infection sites and enhancing host defense-related metabolites.

In addition to improved phosphorus uptake, the higher shoot and root dry weights of mycorrhizal plants may also be associated with enhanced photosynthetic efficiency and improved carbon assimilation during stress which would ultimately lead to biomass increase. Furthermore, AMF colonisation can modify the root architecture and increase root surface area which in turn allows plants to cope better with high influx of plant pathogenic fungi whilst maintaining water and nutrient uptake. Similar observations were made by ChenY. et al AMF inoculation obviously increased tomato tolerance to fungal stress and dry matter accumulation (Jiang et al., 2023).

Statistical analysis showed significant differences (p = 0.00) among treatments which indicate major impacts of both pathogenic infection and mycorrhizal inoculation on tomato growth as shown in table-1,2 respectively. As a whole, the current results indicate that G. mosseae improved tomato growth and antagonized negative impact from F. brachygibbosum infection, it also suggests its potential application as ecologically sound biocontrol agent for Fusrium disease control in tomatoes cultivation system.

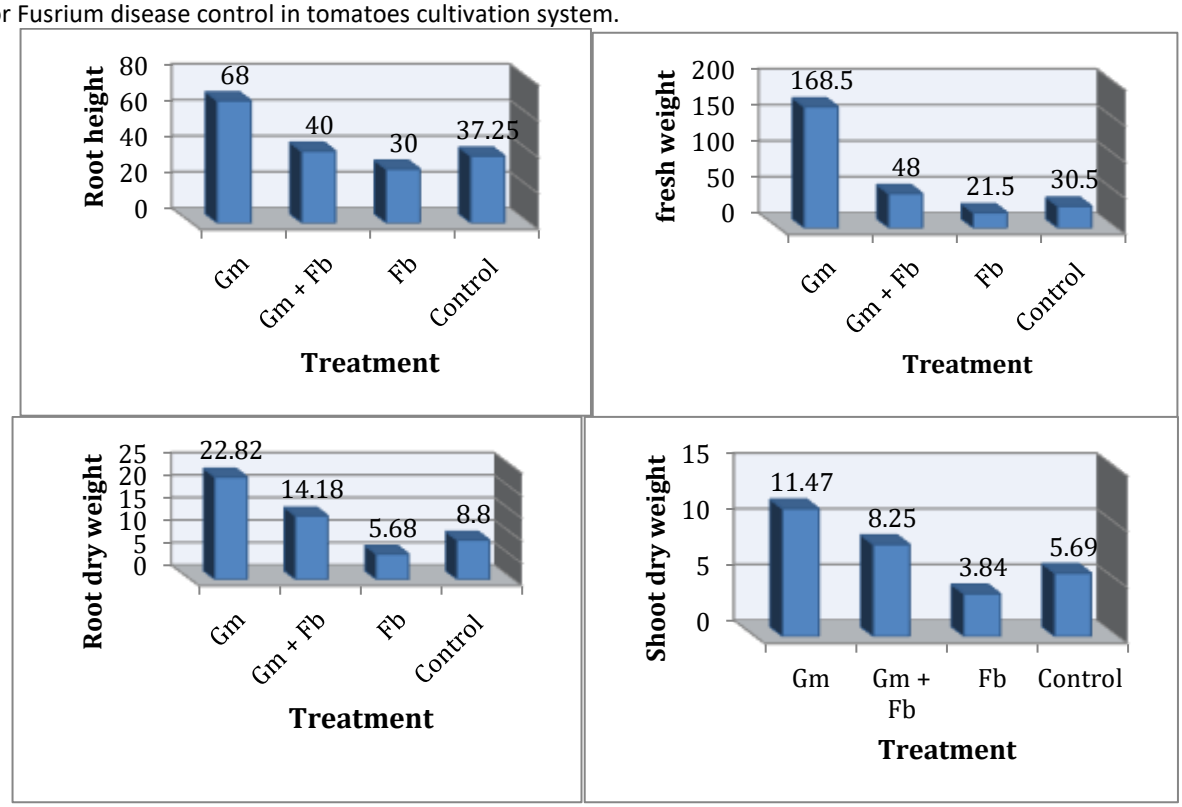
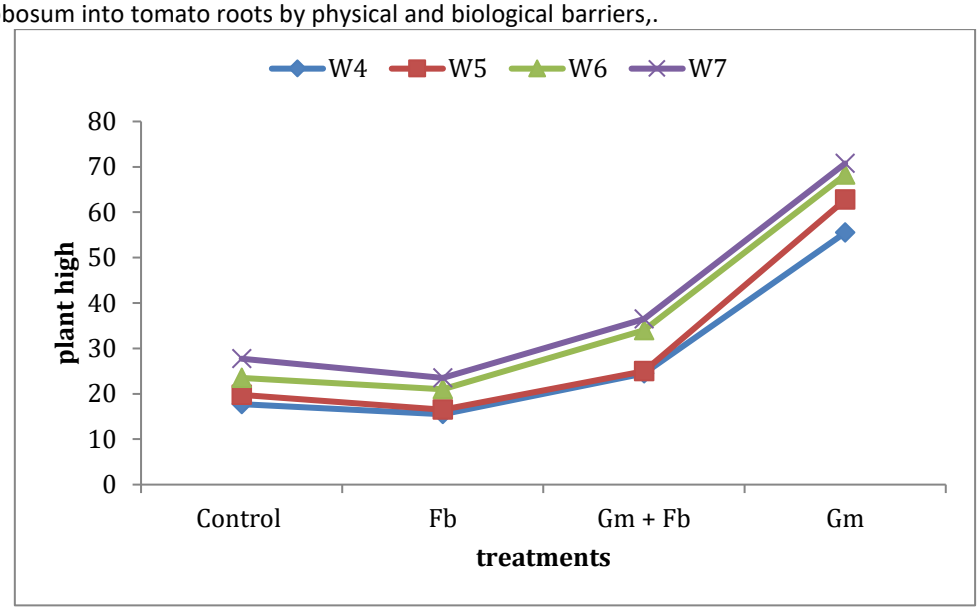


Figure 2: Influence of GM and Fb treatments on plant growth characteristics(fresh weight , Root height, Shoot dry weigh, Root dry weight)which is GM Glomus mosseae and FB Fusarium brachygibbosum

3.3. Pathogenic Suppression of Fusarium brachygibbosum and the "Rescue Effect"

Inoculation with F. brachygibbosum (Fb) led to drastic growth inhibition, where plant height was reduced by 53\% compared to the GM treatment (44.25 cm; Figure 3). This pathogenic effect is characteristic of the Fusarium species, which invade vascular tissues leading to xylem occlusion and vessel blockage due to fungal mycelia and associated phytotoxin synthesis (Ghazal et al., 2022; Tariq et al.2023)

Conversely, dual inoculation significantly ameliorated these symptoms (GM+Fb) with the plant height relative to control recovering up to 77.75 cm. This "Rescue Effect" suggests that G. mosseae outcompetes Fb for infection sites and nutrients in the rhizosphere through a mechanism called niche exclusion (He et al., 2021; Li et al., 2021). Our data corroborate findings of Al-Askar et al. (2023) mentioned that AMF can prevent the entry of F. brachygibbosum into tomato roots by physical and biological barriers,.



(Figure 3). Effect of inoculation with (Gm) and (Fb) on the length of a tomato plant during the growth weeks (weeks 7-10). The vertical bars indicate the standard error, where (Gm) represents the fungus G. mosseae, and Fb represents F. brachygibbosum.

Among treatments there were significant differences in chlorophyll content of tomato plants infected with the soil microbe, Fusarium brachygibbosum ($p \leq 0.05$). However, the highest values of chlorophyll a (3.0380), b (2.89) and total chlorophyll content (524.78) obtained from tomato plant treated by Glomus mosseae GM in(Figure 4). Conversely, plants infected only with F. brachygibbosum had the lowest concentrations of pigments; chlorophyll a (1.1055), chlorophyll b (1.08) and total chlorophylls were measured as 354·82 respectively in these plants The gm + Fb treatment exhibited intermediate values (values between those of control and the other two single treatments), while chlorophyll remained moderately constant in t he control.

The lower chlorophyll content of F. brachygibbosum-infected samples could be due to damaging effects because the pathogen attacks photosynthetic tissues and physiological metabolism. Fusarium infection is often accompanied by oxidative stress, chloroplast injury and damage of photosynthetic enzyme activity, resulting in the decline of chlorophyll pigment levels as well as reduced efficiency We conclude that reduced chlorophyll concentration in infected plants reflects the deleterious effects of pathogen stress on tomato physiology. Researchers found the similar results that tomato plants under biotic stress conditions suffered with Fusarium infection and their chlorophyll content and photosynthesis activity reduced remarkably .

But, inoculation with G. mosseae increased chlorophyll in leaves of tomato plant very much (from other groups). Arbuscular mycorrhizal fungi have enhanced mineral nutrition uptake like nitrogen, magnesium and phosphorus (nutrients required for chlorophyll biosynthesis and photosynthetic processes). In addition, AMF colonization increases the antioxidant defense systems that help protect plants from oxidative damage and improves plant water relations. In the present study, the elevated chlorophyll a, b and total Chloropipped content (Figure 5(a)) of GM plants implies higher photosynthetic potential at high salinity stress likely underlying enhanced drought tolerance in this context. The similar findings were reported by Begum N. et al . (2024) found more chlorophyll biosynthesis and protected photosynthetic pigments from environmental and biotic stress with AMF inoculation in their findings.

In addition, chlorophyll contents of the combined treatment (GM + Fb) were significantly higher than those from the azole and pathogen treatments alone; thus G. mosseae also overcame some deleterious effects imposed by

F. brachygibbosum on plants. This protective effect may be associated with improved nutrient acquisition, stabilization of chloroplast membranes, and activation of plant defense mechanisms induced by AMF colonization. Previous studies demonstrated that mycorrhizal fungi can maintain higher chlorophyll concentrations in pathogen-infected plants by reducing oxidative injury and improving physiological adaptation under stress conditions.

The statistical analysis revealed highly significant differences among treatments for chlorophyll a and chlorophyll b ($p = 0.00$), while total chlorophyll also showed significant variation ($p = 0.009$). The presence of different superscript letters among treatment means further confirms the existence of statistically significant differences. Overall, the present findings indicate that G. mosseae effectively enhanced photosynthetic pigment accumulation and reduced the adverse physiological effects associated with F. brachygibbosum infection in tomato plants.

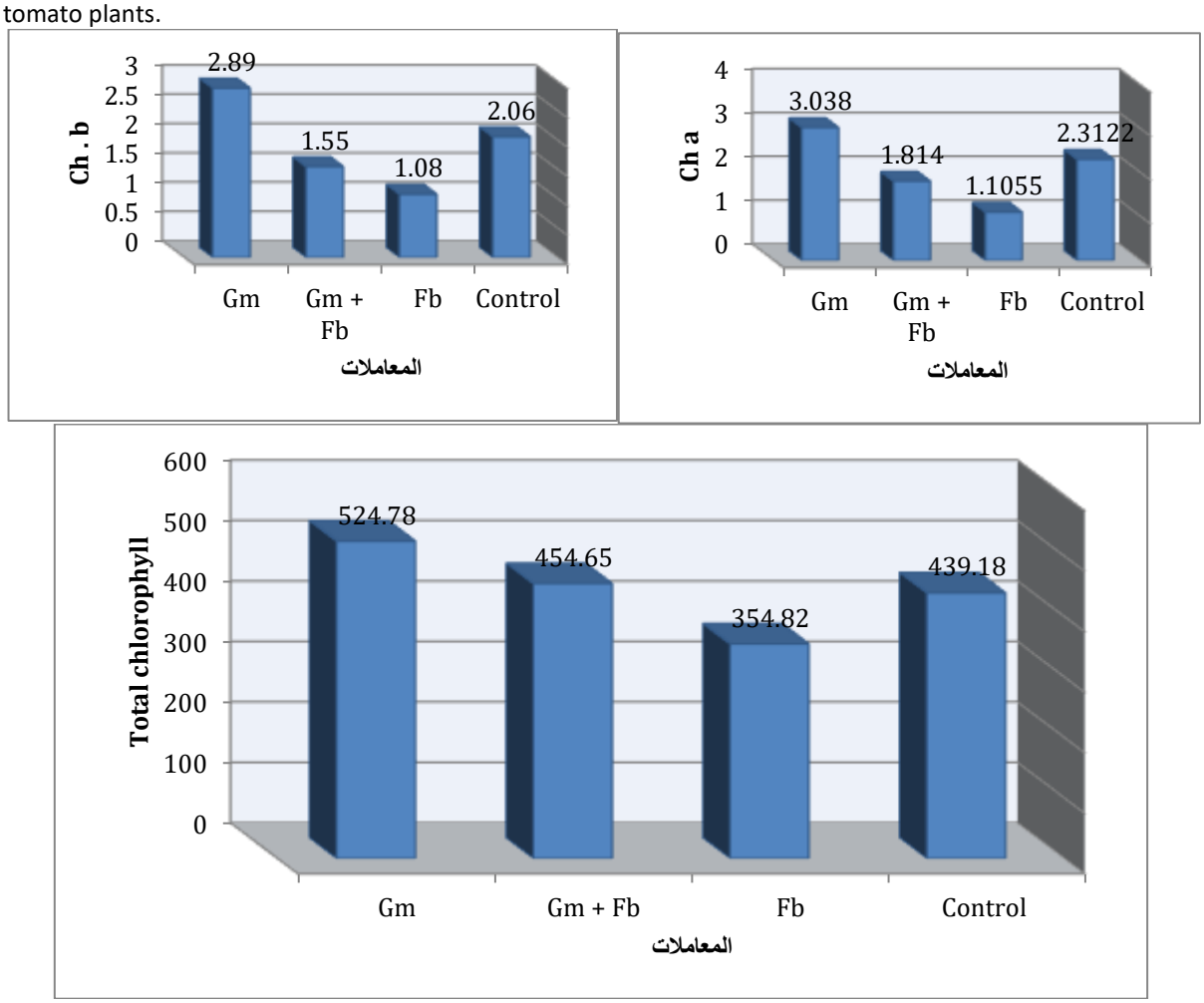


Figure. 4 Effect of (Gm) and (Fb) on chlorophyll (a) (ch.a), chlorophyll (b) (ch.b) and total chlorophyll (Total ch.) found in tomato leaves after 10 weeks of plant growth, while (Gm) G.mosseae, and Fb (F. brachygibbosum)

3.5. Biochemical Defense Priming: Total Phenolics and Lignification

The most striking finding was the synergistic accumulation of Total Phenolic Content (TPC) in the GM+Fb treatment (226.42 mg/g)(Figure 5). This concentration significantly exceeded both the GM-only (189.78 mg/g) and Fb-only (179.62 mg/g) treatments, indicating a potent "Priming Effect" (Mycorrhiza-Induced Resistance, MIR). As proposed by Pozo & Azcón-Aguilar (2007), AMF colonization "pre-activates" the host's immune system, allowing for a faster and more massive response upon pathogen challenge.

These accumulated phenolics serve as precursors for lignin biosynthesis, which structurally fortifies the cell walls against fungal penetration (Akram et al., 2021; Gupta et al., 2022). The high TPC in the GM+Fb group suggests that the plant has shifted its metabolism towards the production of antimicrobial secondary metabolites to inhibit the hyphal branching of F. brachygibbosum (Hoseinzadeh et al., 2020).

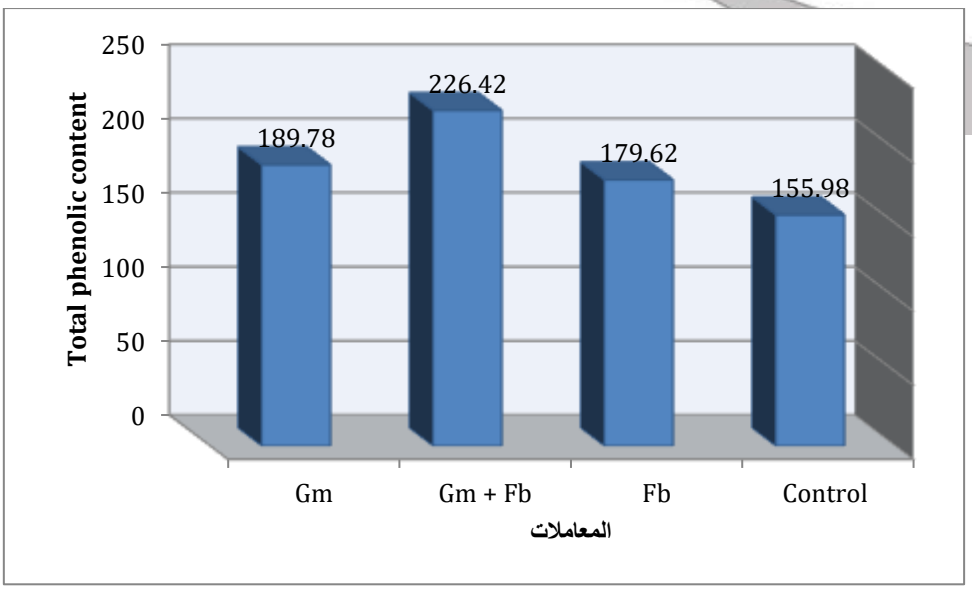


Figure 5. Stimulation of phenolic activity by endogenous treatments against inoculation with the fungus Fusarium brachygibbosum in tomatoes. Vertical bars indicate standard error, and means with different letters differ significantly from each other (p<0.05) according to the LSD test, while (Gm) G. mosseae, and (Fb) Fusarium brachygibbosum.

3.6. Antioxidant Armor: Enzymatic Activities of POD and PPO

The activities of defense enzymes, Peroxidase (POD)(Figure6) and Polyphenol Oxidase (PPO)(Figure 7), Significant differences were observed among treatments in peroxidase (POX) and polyphenol oxidase (PPO) activities in tomato plants infected with Fusarium brachygibbosum ($p \leq 0.05$). The combined treatment of Glomus mosseae and F. brachygibbosum (GM + Fb) recorded the highest enzyme activities, reaching 0.11 for peroxidase and 0.62 for polyphenol oxidase. In contrast, the control treatment exhibited the lowest PPO activity (0.26), while the Fb treatment alone showed the lowest peroxidase activity (0.03).

"The significant increase in POD and PPO activities in the (GM + Fb) treatment aligns with the findings of Yadav et al. (2021) and Song et al. (2024), who reported that mycorrhizal pre-inoculation enhances the enzymatic antioxidant armor of tomato plants against soil-borne pathogens. The synergistic effect observed in our study, where POD reached 0.11 and PPO reached 0.62, confirms the role of Glomus mosseae in priming the plant's defense system, a mechanism also highlighted by Dowarah et al. (2022)."

Plants inoculated with GM alone displayed intermediate but significantly elevated enzyme activities compared with the control.

The increase in peroxidase and polyphenol oxidase activities in the GM + Fb treatment indicates activation of the plant defense system in response to pathogen stress. Peroxidase and PPO are considered important defense-related enzymes involved in lignification, phenolic compound oxidation, reinforcement of cell walls, and detoxification of reactive oxygen species generated during pathogen infection. The enhanced activity of these enzymes suggests that mycorrhizal inoculation stimulated systemic resistance mechanisms in tomato plants against F. brachygibbosum infection.

The high antioxidant enzyme activities in mycorrhizal plants seem to be due to AMF priming plant defense prior pathogen invasion. Mycorrhizal colonization also increases production of defense metabolites, trigger antioxidant pathways and increase plant tolerance to oxidative stress associated with fungal infection [8]. Begum N. et al., also reported similar results. (2024) also highlighted the functional significance of AMF in modulating activity levels across a range of antioxidant enzymes (e.g., peroxidase and polyphenol oxidase) under both biotic and abiotic stress conditions.

The diminished enzyme activities observed in plants infected by F.brachygibbosum alone may reflects the severe physiological disruption or a compromised defense effectiveness that occurs under pathogen stress. Overwhelming oxidative stress induced by Fusarium infection interrupts cellular metabolism and undermine antioxidant defense systems. On the contrary, significant increase in enzyme activities under GM + Fb treatment implicates that G. mosseae augmented tomato resistance and minimized oxidation injury mediated by pathogen.

Polyphenol oxidase activity was most responsive across treatments, especially in the GM + Fb treatment when compared to peroxidase activity. This increase can be associated to the more efficient oxidation of phenolic compounds into toxic quinones that hinder pathogen progression. It has been previously shown that increased PPO activity is directly correlated with the development of induced resistance to Fusarium species in tomato plants [33,34]. Furthermore, the enhancement of PPO activity by AMF is also conducive to strengthening structural and biochemical barriers against pathogen invasion.

The importance of mycorrhizal inoculation on the modulation of antioxidant defense responses in tomato plants under fungal infection stress was confirmed by very highly significant differences among treatments for peroxidase and polyphenol oxidase activities (p = 0.00) as shown from statistical analysis results. In summary, the current results indicate that G. mosseae activates enzymatic antioxidant defense mechanisms to improve tomato resistance against F.brachygibbosum.

The GM + Fb treatment demonstrated a significant increase of enzyme activity, which suggests that G. mosseae ameliorated the defensive ability of tomato plants(Dutta et al.) and (2023)s in a mycorrhizal plant model under biotic stress. Additionally, the moderate defense efficiency found in plants infected only with F. brachygibbosum is indeed consistent with Li et al (2023) illustrates that some Fusarium species are able to manipulate the host antioxidant systems during infection. The superior response of PPO in our study is additionally reinforced by Amanifar & Toghranegar (2024), which highlights the activity role this enzyme plays during converting

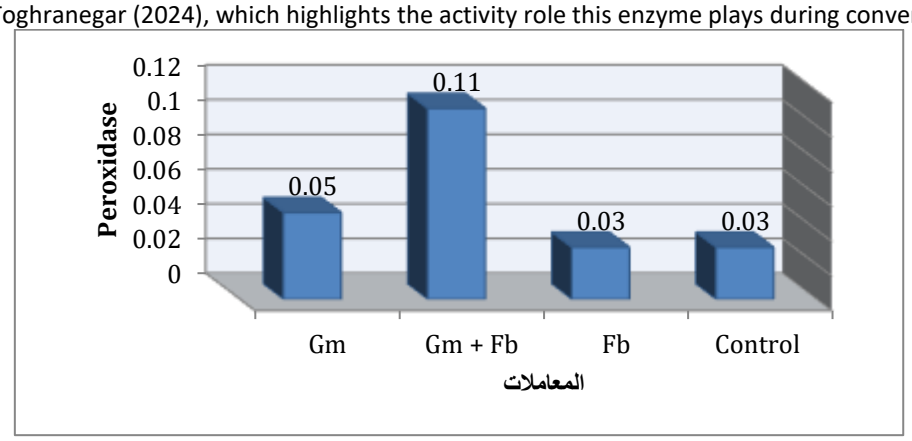


Figure 6. Stimulation of peroxidase activity by different treatments against inoculation with the fungus Fusarium brachygibbosum. Mean peroxidase activity (ΔA420 min-1 g). Vertical bars indicate standard error. Means labeled with different letters differ significantly from each other (p<0.05) according to the LSD test, where (Gm) is G. mosseae, and (Fb) is Fusarium brachygibbosum.

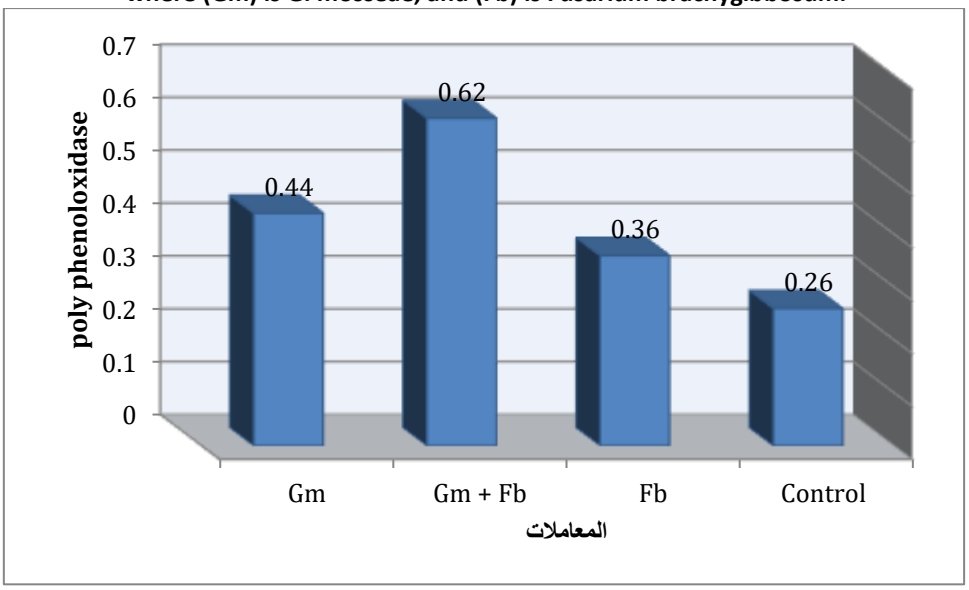


Figure 7. Stimulation of polyphenol oxidase enzyme activity by different treatments against inoculation with the fungus Fusarium brachygibbosum. Mean polyphenol oxidase enzyme activity (µg catechol/g fresh tissue). Vertical bars indicate standard error. Means with different letters differ significantly from each other (p<0.05) according to the LSD test, where (Gm) is G. mosseae fungus, and (Fb) is Fusarium brachygibbosum.

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