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TRICHODERMA HARZIANUM MITIGATES THE PHYSIOLOGICAL AND BIOCHEMICAL STRESS INDUCED BY THE COTTON MEALYBUG *PHENACOCCUS SOLENOPSIS* IN TOMATO

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Abstract

Phenacoccus solenopsis Tinsley, commonly known as the invasive cotton mealybug, is a serious pest that suppresses the growth and physiology of tomato (*Solanum lycopersicum*). This study assessed the role of an entomopathogenic fungal endophyte (*Trichoderma harzianum*) in alleviating mealybug-induced stress under controlled growth-chamber conditions. Four treatments were compared, which included control, mealybug infestation, *T. harzianum* inoculation, and mealybug + *T. harzianum*. Mealybug infestation significantly decreased the height of plants (29.8 cm), root depth (18.1 cm), shoot dry weight (2.76 g), root dry weight (1.03 g), and total pigment content (1.20 mg g^{-1} fresh weight, FW) and increased proline (70.65 µmol g^{-1} FW), glycine betaine (13.43 µg g^{-1} FW), malondialdehyde (MDA, 7.43 µmol g^{-1} FW) and hydrogen peroxide (H_2O_2, 7.74 µmol g^{-1} FW). In contrast, *T. harzianum* treatment alone maximized growth (plant height: 86.83 cm; root depth: 48.87 cm; shoot dry weight: 7.68 g; root dry weight: 3.38 g), pigment content (2.14 mg g^{-1} FW), and relative water content (92.21%), with minimal oxidative stress (MDA: 4.05 µmol g^{-1} FW; H_2O_2: 3.34 µmol g^{-1} FW). Co-application partially restored growth (plant height: 52.67 cm; shoot dry weight: 4.80 g), and decreased stress markers (MDA: 5.17 µmol g^{-1} FW; H_2O_2: 4.90 µmol g^{-1} FW), while further raising proline (88.04 µmol g^{-1} FW) and glycine betaine (18.05 µg g^{-1} FW). Infestation also raised the activities of indole-3-acetic acid oxidase (IAA oxidase, +45%) and polyphenol oxidase (PPO, +142%), whereas co-inoculation lowered both and produced the highest activities of superoxide dismutase (16.80 U mg^{-1} protein), catalase (10.27 U mg^{-1} protein), ascorbate peroxidase (13.94 U mg^{-1} protein) and glutathione reductase (2.25 U mg^{-1} protein), 17 to 26% above those of infested plants. One-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) test and principal component analysis (PCA) indicated significant treatment effects ($p<0.001$) and clear separation of the four treatments, each represented by three biological replicates. These findings indicate that *T. harzianum* can help tomato plants tolerate mealybug attack by sustaining growth, photosynthetic pigments, water status and redox balance, in association with a stronger antioxidant enzyme system and restrained auxin-degrading activity. Because mealybug population size, survival and fecundity were not recorded, these results describe improved host tolerance to infestation rather than demonstrated suppression of the pest, and any contribution of this endophyte to integrated pest management remains to be established in insect-performance and field studies.

Key words: *Phenacoccus Solenopsis*; Tomato; *Trichoderma harzianum*; Entomopathogenic endophytes; Induced tolerance; Stress mitigation; Oxidative stress; Osmoprotectants; Antioxidant enzymes

Introduction

Plant-microorganism-insect pest complex interaction has a significant impact on agricultural sustainability and crop productivity. Among them, the interactions between plants and fungi are the most important in the regulation of the health of plants, their growth, and ecology. Such interactions can be mutually beneficial symbioses, e.g., mycorrhizal associations, which increase nutrient and water uptake, or detrimental relationships, e.g., pathogenic fungi resulting in severe crop diseases and economic losses (Bennett & Cahill, 2016; Kastner *et al.,* 2021). Fungi also play a role in the process of nutrient cycling and ecosystem stability during decomposition and nutrient recycling in natural and agricultural ecosystems, thus determining the plant diversity and productivity (Frąc *et al.,* 2018). However, fungal pathogens can lead to substantial yield reductions, affecting various plant organs and impairing physiological functions such as photosynthesis (Chatterjee *et al.,* 2016).

In response to these difficulties, plants have developed complex innate immune systems which include constitutive and inducible defense systems. They comprise structural mechanisms, such as the cell wall and the cuticle, and biochemical mechanisms, such as phytoalexins, reactive oxygen species (ROS), and pattern recognition receptor (PRR)-mediated signalling (Bigeard *et al.,* 2015; Riaz *et al.,* 2021). When plants sense the presence of pathogen-associated molecular patterns (PAMPs), they trigger complex cascades of signalling, such as mitogen-activated protein kinase (MAPK) signalling, resulting in increased defense responses (Tripathi *et al.,* 2024). Moreover, salicylic acid (SA) and jasmonic acid (JA) hormonal pathways control the plant reaction to biotrophic pathogens and chewing insects, respectively (Gupta *et al.,*

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2020). Phloem-feeding insects such as mealybugs are particularly successful because they can exploit the antagonism between these two pathways, activating SA signaling while suppressing the JA-dependent defenses that would otherwise limit their performance (Zhang *et al.,* 2015). Irrespective of these defenses, plant immunity is constantly being outmaneuvered by pathogens and pests, and a co-evolutionary arms race occurs (Zhang *et al.,* 2022; Tripathi *et al.,* 2024).

Insect pests are also a significant biotic limitation in agriculture; both direct damage by feeding and indirect losses by spreading plant pathogens. Among them, sap-sucking insects are the most dangerous since they reproduce quickly and can utilize the resources of plants effectively. One such invasive and polyphagous pest is the cotton mealybug, *Phenacoccus solenopsis* Tinsley, which has a substantial list of crops that it can infest, which includes tomato, cotton, and potato (Fand and Suroshe, 2015; El Aalaoui *et al.,* 2024; Namadara *et al.,* 2025). This pest destroys plants by sucking the sap, releasing honeydew, which encourages the development of sooty molds, and injecting harmful substances, which cause stunted growth, wilting, and even death of the plants (Tong *et al.,* 2019). It is very difficult to control with the help of traditional methods due to its high reproductive capacity and the possibility of having several generations annually (Arif *et al.,* 2012). Synthetic pesticides are widely applied, but they are not always effective because the pest has a waxy surface and cryptic behavior, and they are excessively used, which results in the development of resistance and contaminates the environment and disrupts natural predator communities (Nawaz & Freed, 2022).

In that regard, biological control methods have been a subject of growing interest as natural alternatives to chemical pesticides. Entomopathogenic fungi (EPF) belong to the most promising biological control agents because of their ability to infect and cause the death of a large number of insect pests. These fungi infect insects by direct cuticle penetration and then internal growth and release of toxic metabolites that eventually lead to the death of the host (Sharma & Sharma, 2014; El Aalaoui *et al.,* 2024; Ma *et al.,* 2024). A considerable percentage of insect diseases are natural and caused by EPF, which are environmentally friendly because they are specific and have fewer impacts on non-target organisms (Litwin *et al.,* 2020). Beyond classical entomopathogens such as *Beauveria* and *Metarhizium*, species of *Trichoderma*, long known as mycoparasites and plant growth promoters, are increasingly recognized for their activity against insect pests, which they exert through insecticidal and antifeedant metabolites and, indirectly, through the induction of plant defenses (Poveda, 2021; Woo *et al.,* 2023).

In addition to their direct insecticidal action, EPF may also become endophytes in plant tissues, where they develop entomopathogenic endophytic fungi (EEPF). These endophytes do not cause damage but colonize plant tissues and indirectly repel herbivorous insects by increasing plant defense mechanisms and bioactive compounds (Samal *et al.,* 2023). EEPFs also play a role in complicated tritrophic interactions with plants, insects, and fungi, and can transfer nutrients, alter plant metabolism, and enhance plant resistance (Behie *et al.,* 2017). In addition, fungal-derived molecules, including chitin, β-glucans, and other microbe-associated molecular patterns (MAMPs), are capable of inducing systemic resistance in plants, such as systemic acquired resistance (SAR) and induced systemic resistance (ISR), and enhance plant resistance to pests (Nishad *et al.,* 2020; Vlot *et al.,* 2021). In tomato, root colonization by *Trichoderma* has been shown to strengthen both direct and indirect defenses against aphids and lepidopteran larvae (Coppola *et al.,* 2019) and to modulate defense responses to stink bug feeding (Alınç *et al.,* 2021), largely by priming JA-regulated defenses (Martínez-Medina *et al.,* 2017). Under insect attack, plants also depend on antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase to keep reactive oxygen species within safe limits (Gill and Tuteja, 2010; Kerchev *et al.,* 2012), while oxidative enzymes such as polyphenol oxidase contribute directly to anti-herbivore defense (Bhonwong *et al.,* 2009).

The widespread deployment of EPF faces various challenges, such as environmental sensitivity, low shelf life, and variability in field performance, despite its positive aspects (Zhao *et al.,* 2016; Mascarin *et al.,* 2019; Valencia *et al.,* 2025). Also, the relationships between EPF, host plants, and insect pests are extremely complicated and determined by various factors, such as plant genotype, environment, and dynamics of microbial communities (Darsouei *et al.,* 2024). As a result, a better understanding of these associations is required to optimize the application of EPF in integrated pest management (IPM) systems.

Due to the rising worldwide demand for food and the constraints of traditional pest control methods, there is an urgent need to develop sustainable and environmentally friendly alternative methods for insect pest management (Etesami & Jeong, 2018; Samal *et al.,* 2023). In this regard, exploring the use of entomopathogenic fungal endophytes as dual-functional agents capable of directly reducing insect pests while indirectly increasing plant defense is a viable route for increasing yields from agriculture and resilience.

The present study aims to investigate the role of entomopathogenic fungal endophyte (*Trichoderma harzianum*) in mitigating the impact of *Phenacoccus solenopsis* on tomato plants (*Solanum lycopersicum* L.), with a particular focus on its influence on plant growth, physiological, and biochemical responses, including the activities of oxidative and antioxidant enzymes. We hypothesized that colonization by *T. harzianum* would help infested plants maintain growth, photosynthetic pigments, water status and redox balance, supported by a stronger antioxidant enzyme system and lower auxin-degrading activity, thereby reducing the physiological cost of mealybug infestation.

Materials and Methods

Plant material: Certified healthy seeds of tomato (*Solanum lycopersicum* L. cv. Newton, syn. *Lycopersicon esculentum* Mill.), supplied by Syngenta Vegetable Seeds, Switzerland, were used in the current study. All experiments were carried out on two-week-old seedlings, which were transplanted into pots and maintained in a growth chamber as described in Section 2.4.

Insect material: An adult cotton mealybug culture of *Phenacoccus solenopsis* was collected from infested tomato plants grown in Al Kharj, Riyadh, Saudi Arabia by Professor Abeer Hashem (Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia) who carried out the molecular identification previously and recorded it in GenBank under accession number: PZ639065. The insects were reared under controlled laboratory conditions as described by Abdullah and El-Rokh (2023). The propagation and inoculation of tomato plants with *P. solenopsis* were carried out according to the method described by El Aalaoui *et al*., (2024). Adult males and females of *P. solenopsis* on infested tomato plants, and the honeydew they secrete, are shown in Figures 1 and 2 respectively.

Entomopathogenic fungal endophyte: The entomopathogenic fungal isolate (*Trichoderma harzianum*, GenBank accession number: PP762175) was previously isolated and identified by Professor Abeer Hashem (unpublished data). The isolate was maintained on potato dextrose agar medium (PDA, Booth, 1977). Identification of the endophytic biocontrol agent was carried out according to Barnett & Hunter (1987) and Goettel and Inglis (1997) based on spore and colony morphology, with additional confirmation via ITS region sequencing (GenBank accession number: PP762175). The entomopathogenic potential of the experimental biocontrol agent *T. harzianum* was confirmed *In vitro* and *In vivo* (unpublished data).

Experimental design and growth chamber trials: A completely randomized design (CRD) was used to evaluate the efficacy of the fungal endophyte *T. harzianum* in alleviating the stress caused by *P. solenopsis* in tomato plants. The study was conducted in a growth chamber under controlled conditions (temperature, 28 ± 2°C; 65% relative humidity; and a fluorescent lighting system providing an 18 h light / 6 h darkness photoperiod) in the Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia (Female branch). Tomato seedlings (two weeks after germination) were transplanted into plastic pots (25 cm diameter, 18 cm height, one plant per pot) filled with a mixture of clay soil and sphagnum peat moss, following the protocol of Goettel & Inglis (1997). *T. harzianum* was first applied to the soil as an aqueous conidial suspension (1×10^8 conidia mL^{-1} in sterile distilled water containing 0.02% (v/v) Tween 80; 5 mL per pot), delivered as a drench to the root zone one week before mealybug infestation (Hashem *et al.,* 2014; Xiong *et al.,* 2018). Adult mealybugs of *P. solenopsis* were inoculated onto tomato plants seven days after soil treatment with *T. harzianum* (five adult females per plant) as described by Panyasiri *et al*., (2007) and El Aalaoui *et al*., (2024). The insects were allowed to settle for 48 h, after which tomato plants were sprayed to run-off with the same conidial suspension of *T. harzianum* (1×10^8 conidia mL^{-1}; 5 mL per plant) following Csótó *et al*., (2024). Conidial concentrations were determined with a haemocytometer. Control and mealybug-only plants received the same volumes of sterile distilled water containing 0.02% (v/v) Tween 80, by the same routes and on the same dates, as matched carrier controls. The pots were incubated for 8 weeks after treatments. The study consisted of 4 treatments: 1: Plants without any treatment (control); 2: Plants infested with adult cotton mealybug (*P. solenopsis*); 3: Plants exposed to both cotton mealybug and fungal endophyte (*T. harzianum*); 4: Plants inoculated with entomopathogenic fungal endophyte. Each treatment comprised 3 independent biological replicates (n = 3 pots, one plant per pot), and the pots of all 4 treatments were randomized within the growth chamber and re-randomized weekly. Plants were collected at the end of the experiment and tested for growth, physiological, and biochemical characteristics. Representative plants of each treatment were photographed at harvest (Fig. 3).

Determination of plant growth parameters: The lengths of the shoots (plant height, PH) and roots (root depth, RD) were measured using a ruler. Fresh weights (FW) of shoots and roots were calculated immediately, whereas dry weights (shoot dry weight, SDW; root dry weight, RDW) were measured after 72 hours of oven drying at 70°C.

Photosynthetic pigment analysis: Chlorophyll content in leaves was extracted using dimethyl sulfoxide (DMSO) and quantified spectrophotometrically following Lichtenthaler (1987). Chlorophyll a, chlorophyll b and carotenoid concentrations were calculated with the equations of Lichtenthaler (1987) and expressed as mg g^{-1} fresh weight.

Estimation of osmolytes: Proline concentration was measured according to Bates *et al*., (1973) with absorbance read at 520 nm against a toluene blank, and expressed as µmol g^{-1} FW. Glycine betaine (GB) content was estimated following Grieve and Grattan (1983) with absorbance measured at 365 nm, and expressed as µg g^{-1} FW.

Relative water content: Leaf relative water content (RWC) was determined following Barrs & Weatherley (1962) and calculated as RWC (%) = [(FW − DW)/(TW − DW)] × 100, where FW, TW and DW are the fresh, turgid and oven-dry weights of the leaf samples, respectively.

Oxidative stress and lipid peroxidation: Hydrogen peroxide (H_2O_2) content was determined using Velikova *et al*., (2000) by recording the absorbance at 390 nm, and expressed as µmol g^{-1} FW. Lipid peroxidation (malondialdehyde, MDA) was analyzed following Heath & Packer (1968). Absorbance was measured at 532 nm and corrected for non-specific absorbance at 600 nm, with 1% thiobarbituric acid in 20% trichloroacetic acid as the blank; MDA content was expressed as µmol g^{-1} FW.

Assay of oxidative and antioxidant enzymes: For the enzyme assays, fresh leaf tissue was homogenized in ice-cold potassium phosphate buffer and centrifuged, and the clear supernatant was used as the enzyme extract. Soluble protein in the extract was determined by the dye-binding method of Bradford (1976) with bovine serum albumin as the standard, and all enzyme activities were expressed as enzyme units per mg of protein (U mg^{-1} protein), the unit

being defined below for each assay. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed from its ability to inhibit the photochemical reduction of nitroblue tetrazolium, measured at 560 nm (Beauchamp & Fridovich, 1971). One unit of SOD activity is the amount of enzyme required to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) by 50% under the specific assay conditions. Catalase (CAT, EC 1.11.1.6) activity was determined from the decomposition of H_2O_2 followed at 240 nm (Aebi, 1984). One unit of CAT activity is defined as µmol H_2O_2 min^{-1} mg^{-1} protein. Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured from the decrease in absorbance at 290 nm caused by the oxidation of ascorbate (Nakano and Asada, 1981). One unit of APX activity is defined as µmol ascorbate oxidized min^{-1} mg^{-1} protein. Glutathione reductase (GR, EC 1.8.1.7) activity was estimated from the oxidation of NADPH at 340 nm in the presence of oxidized glutathione (Foyer and Halliwell, 1976). One unit of GR activity is defined as µmol NADPH oxidized min^{-1} mg^{-1} protein. Polyphenol oxidase (PPO, EC 1.10.3.1) activity was assayed with catechol as the substrate by following the increase in absorbance at 420 nm (Mayer *et al.,* 1966). One unit of PPO activity is defined as ΔA_{420} min^{-1} mg^{-1} protein. Indole-3-acetic acid oxidase (IAA oxidase) activity was determined from the amount of IAA destroyed during incubation of the enzyme extract with IAA, the residual IAA being quantified colorimetrically with the Salkowski reagent at 530 nm (Gordon and Weber, 1951; Beffa *et al.,* 1990). One unit of IAA oxidase activity is defined as µg IAA decomposed min^{-1} mg^{-1} protein.

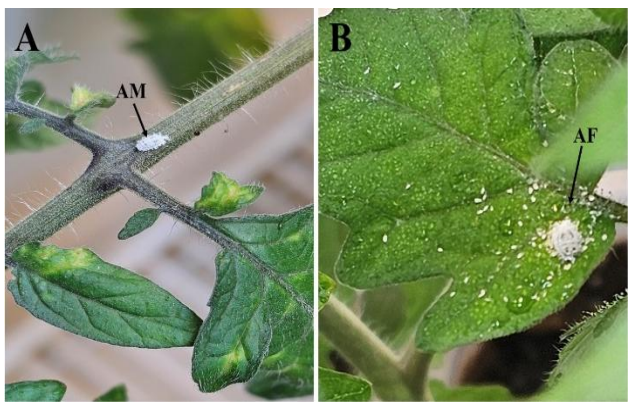


Fig. 1. Adult cotton mealybugs (*Phenacoccus solenopsis*) on infested tomato plants: **A**, adult male (AM, arrow); **B**, adult female (AF, arrow). Original magnification 1.4×. Photographs were taken by Dalal Saad Alharbi in the growth chamber at King Saud University, Riyadh, Saudi Arabia.



Fig. 2. Honeydew secretions (HS, arrow) produced by the cotton mealybug (*P. solenopsis*) on tomato plants. Original magnification 1.4×. Photographs were taken by Dalal Saad Alharbi in the growth chamber at King Saud University, Riyadh, Saudi Arabia.

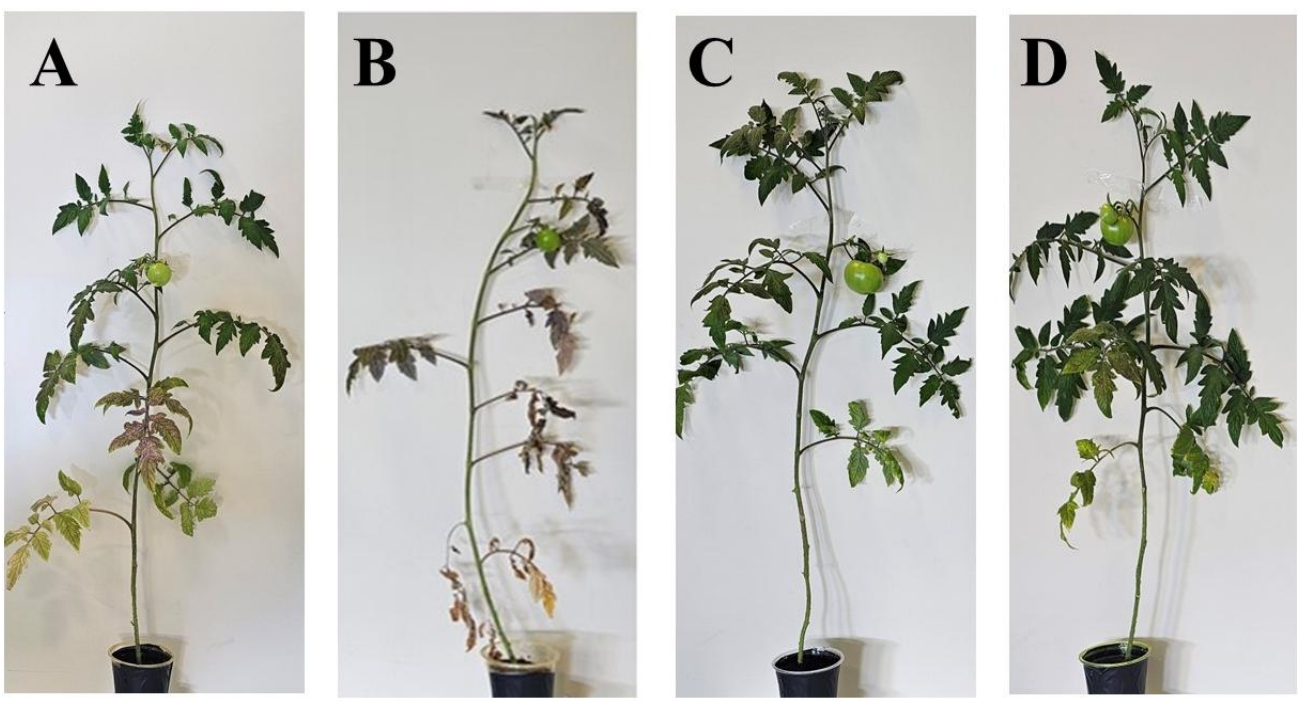


Fig. 3. Phenotypic response of tomato plants to cotton mealybug (*Phenacoccus solenopsis*) infestation and to inoculation with *Trichoderma harzianum*, eight weeks after treatment. **A,** plant control**; B,** plant + *P. solenopsis***; C,** plant + *T. harzianum* + *P. solenopsis***; D,** plant + *T. harzianum***.** Photographs were taken by Dalal Saad Alharbi in the growth chamber at King Saud University, Riyadh, Saudi Arabia.

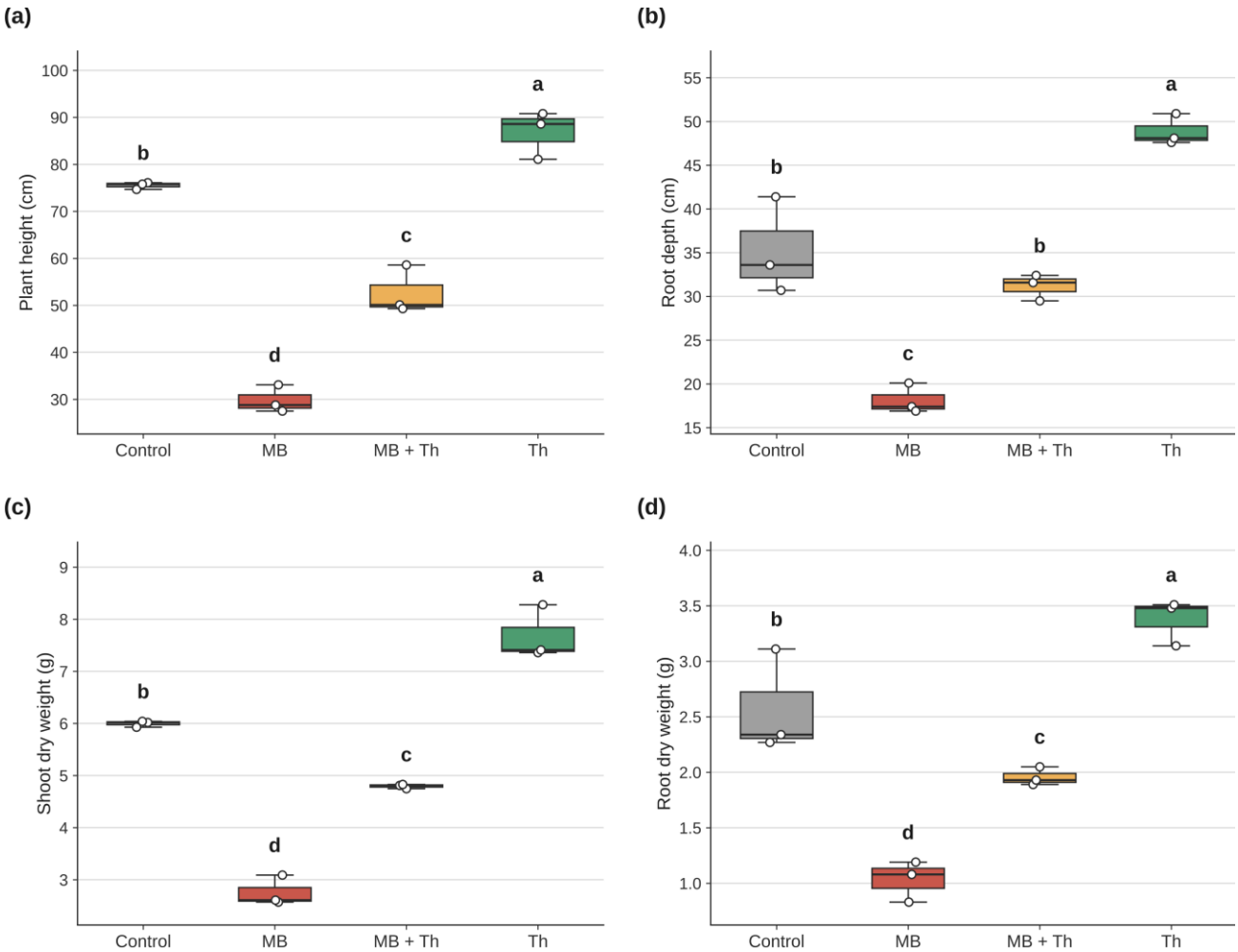


Fig. 4. Growth parameters under different treatments. Boxplots showing the effect of treatments (Plant Control, Cotton Mealybug, Mealybug + *Trichoderma*, and *Trichoderma* alone) on plant growth attributes including (a) plant height (PH), (b) root depth (RD), (c) shoot dry weight (SDW), and (d) root dry weight (RDW). Each panel represents an individual parameter. Data points indicate biological replicates (n = 3), with boxplots illustrating median, interquartile range, and variability among treatments. Different letters above the boxes indicate significant differences among treatments (the least significant difference (LSD) test, $p \leq 0.05$). MB, cotton mealybug; Th, *T. harzianum*.

Statistical analysis

The experiment followed a completely randomized design with 4 treatments and 3 biological replicates per treatment (n = 3). Each pot was treated as the experimental unit. Treatment means are given in Tables 1 to 6, and the distribution of the replicate values is shown as boxplots in Figures 4 to 8. All reported values are means of 3 independent biological replicates; the consequences of this limited replication are considered in Section 3.11.

Before analysis, each variable was checked against the assumptions of analysis of variance. Normality of the model residuals was assessed with the Shapiro–Wilk test (Shapiro and Wilk, 1965), and homogeneity of variances among treatments with Levene's test centred on the median (the Brown–Forsythe modification), which is robust to departures from normality (Levene, 1960; Brown and Forsythe, 1974; Fox and Weisberg, 2019). Both assumptions were met for all variables ($p>0.05$) except polyphenol oxidase, whose residuals departed slightly from normality (Shapiro–Wilk $p = 0.048$). PPO data were therefore $\log_{10}$-transformed before analysis (Gomez and Gomez, 1984), which restored normality ($p = 0.46$); all other variables were analysed untransformed. All inferential tests on PPO, that is the ANOVA and the subsequent mean separation, were carried out on the log10-transformed values, whereas the PPO means presented in Table 5 and Fig. 8 are given in the original units (U mg^{-1} protein) for ease of interpretation; the significance letters attached to PPO therefore refer to the analysis of the transformed data.

Treatment effects were tested by one-way analysis of variance (ANOVA) using the linear model $Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$, where Y_{ij} is the observation on the j^{th} replicate of the i^{th} treatment, μ is the overall mean, τ_i is the effect of the i^{th} treatment and ε_{ij} is the random error (Gomez & Gomez, 1984; Steel *et al.,* 1997). When the F-test was significant at $p \leq 0.05$, the treatment means in Tables 1 to 6 and in Figures 4 to 8 were separated with Fisher's least significant difference (LSD) test at $p \leq 0.05$ (Fisher, 1935), calculated as $LSD0.05 = t0.025(df\ error) \times \sqrt{(2MSE/r)}$, where t is the two-tailed Student's t value at the error degrees of freedom (df = 8), MSE is the error mean square of the ANOVA and r is the number of replicates (r = 3). The LSD was applied only after a significant F-test, that is as a protected LSD, and was adopted as the single mean-comparison procedure

throughout the manuscript; the LSD value for each variable is reported in the corresponding table so that any pair of treatment means can be compared directly (Fisher, 1935; Gomez & Gomez, 1984; Steel *et al*., 1997). Means that share a letter within a table or figure do not differ significantly at p≤0.05, and every statement of significance in the Results was checked against the unrounded output of this single analysis. Where the F-test was not significant (the PH/RD, SDW/RDW and chlorophyll a/b ratios), no mean separation was carried out and the comparison is reported as not significant (NS).

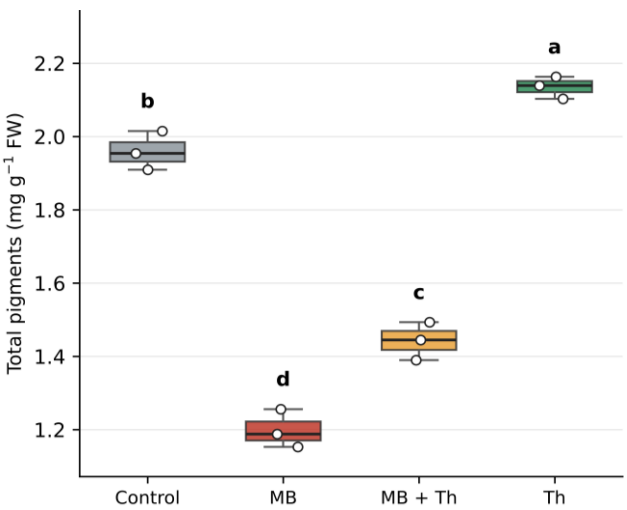


Fig. 5. Total pigment content. Boxplot showing the effect of treatments (Plant Control, Cotton Mealybug, Mealybug + *Trichoderma*, and *Trichoderma*) on total pigment content. Data points represent individual replicates (n = 3), and the boxplot indicates median values and dispersion across treatments. Different letters above the boxes indicate significant differences among treatments (the least significant difference (LSD) test, p ≤ 0.05). MB, cotton mealybug; Th, *T. harzianum*.

Linear associations among growth, photosynthetic, osmotic and oxidative stress traits and enzyme activities were quantified with Pearson's product–moment correlation coefficient (r) (Pearson, 1895), calculated on the pooled individual observations (n = 12; four treatments × three replicates). The significance of each coefficient was tested with a two-tailed t-test at p≤0.05 and p≤0.01, and the correlation matrix was displayed as a heatmap (Fig. 9).

To summarize the multivariate response and examine how treatments clustered, principal component analysis (PCA) was performed on the correlation matrix, that is, on variables standardized to zero mean and unit variance, so that traits measured in different units contributed equally to the analysis (Jolliffe & Cadima, 2016). The analysis was run on the 16 mutually independent traits (Tables 1 to 6), namely plant height, root depth, shoot dry weight, root dry weight, total pigment content, proline, glycine betaine, relative water content, malondialdehyde, hydrogen peroxide, IAA oxidase, polyphenol oxidase, superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase. Chlorophyll a, chlorophyll b and carotenoids were represented in the ordination by total pigment content, and the derived PH/RD, SDW/RDW and chlorophyll a/b ratios were omitted, because entering a variable alongside its own components or ratios would weight those traits twice. Components with eigenvalues greater than 1 were considered meaningful (Kaiser, 1960), and the first two components were used to construct a biplot of treatment scores and variable loadings (Fig. 10).

All statistical analyses and graphics were produced in R version 4.4 (R Core Team, 2024). ANOVA and the LSD test were performed with the agricolae package (de Mendiburu, 2023), Levene's test with the car package (Fox and Weisberg, 2019), boxplots with ggplot2 (Wickham, 2016), the correlation heatmap with corrplot (Wei and Simko, 2024), and PCA with FactoMineR (Lê *et al.,* 2008) and factoextra (Kassambara & Mundt, 2020). Statistical significance was accepted at p≤0.05 throughout.

Results and Discussion

Effect of *Trichoderma* and cotton mealybug infestation on plant growth parameters: Different treatments had a substantial effect on plant development metrics (p<0.001; Table 1, Fig.4). The phenotypic differences among the 4 treatments at harvest are shown in Fig. 3. Control plants grew moderately, with an average plant height of 75.53 cm, root depth of 35.23 cm, and dry biomass (SDW: 5.99 g; RDW: 2.57 g). In contrast, cotton mealybug infestation significantly decreased plant height (29.80 cm), root depth (18.13 cm), and biomass (SDW: 2.76 g; RDW: 1.03 g). However, *Trichoderma* application to mealybug-infested plants increased growth (PH: 52.67 cm; RD: 31.17 cm; SDW: 4.80 g; RDW: 1.96 g), suggesting stress reduction. The highest growth was recorded in *Trichoderma*-treated plants alone, with maximum plant height (86.83 cm), root depth (48.87 cm), and biomass (SDW: 7.68 g; RDW: 3.38 g). Relative to the control, infestation reduced plant height by 60.5%, root depth by 48.5%, and shoot and root dry weights by 54.0% and 59.8%, respectively, whereas in co-inoculated plants these losses were limited to 30.3%, 11.5%, 20.0% and 24.0%. Root depth of co-inoculated plants did not differ significantly from the control, whereas their root dry weight remained significantly below it (Table 1). In contrast, the PH/RD and SDW/RDW ratios were not significantly affected by any treatment, indicating that infestation and fungal inoculation changed overall plant size rather than the allocation of biomass between shoots and roots. These findings aligned with reports where *T. harzianum* improved seedling vigour and growth parameters in cotton under laboratory and field conditions (Sain *et al.,* 2023). Similar growth-promoting effects of *Trichoderma* and entomopathogenic fungi have been observed in strawberry and tomato under biotic stress (Zinan *et al.,* 2025; Wahdat *et al.,* 2025).

The growth penalty caused by *P. solenopsis* is consistent with its feeding biology. Continuous removal of phloem sap drains photoassimilates and amino acids away from growing tissues, while salivary secretions, honeydew and the associated sooty mould further reduce the photosynthetically active leaf area (Huang *et al.,* 2013; Tong *et al.,* 2019). The better root development of *T. harzianum*-treated plants, both with and without the pest, agrees with the well-documented ability of *Trichoderma* to stimulate root growth through auxin-like compounds, the solubilization of phosphate and micronutrients, and secondary metabolites that act as growth regulators (Woo

et al., 2023). A deeper and heavier root system would be expected to improve the capture of water and nutrients, and this offers one plausible explanation for the greater biomass retained by co-inoculated plants relative to infested plants. Because mealybug numbers, survival and fecundity were not recorded in either treatment, the two groups cannot be assumed to have carried an equivalent pest load, and improved water and nutrient uptake remains a hypothesis rather than a measured outcome of the present experiment. Comparable benefits have been reported in tomato colonized by *Trichoderma* and exposed to aphids and lepidopteran larvae, where the fungus sustained plant performance while priming defense pathways (Coppola *et al.,* 2019), and in a range of other crop and pest systems were reviewed by Poveda (2021).

Effect of *Trichoderma* and cotton mealybug infestation on photosynthetic pigments: Photosynthetic pigments were significantly affected by different treatments (Table 2, Fig. 5). Control plants showed moderate pigment content with mean chlorophyll a (1.192 mg g^{-1} FW), chlorophyll b (0.491 mg g^{-1} FW), and total pigments (1.96 mg g^{-1} FW). Cotton mealybug infestation markedly reduced all pigment levels, with chlorophyll a (0.748 mg g^{-1} FW), chlorophyll b (0.295 mg g^{-1} FW), and total pigments (1.20 mg g^{-1} FW), indicating impaired photosynthetic capacity. However, *Trichoderma* application in infested plants improved pigment content (total pigments: 1.44 mg g^{-1} FW), reflecting partial recovery. *Trichoderma*-treated plants had the largest pigment accumulation, with the highest chlorophyll a (1.305 mg g^{-1} FW), chlorophyll b (0.518 mg g^{-1} FW), and total pigments (2.14 mg g^{-1} FW). Carotenoids followed the same trend, whereas the chlorophyll a/b ratio did not differ significantly among treatments (Table 2), suggesting that infestation reduced the whole pool of light-utilizing pigments rather than only chlorophyll form selectively. These results are consistent with prior research, which has shown that mealybug infestation lowers chlorophyll a, chlorophyll b, and total chlorophyll in cotton (Prabhakar *et al.,* 2013; Nagrare *et al.,* 2017) and other host plants such as tomato (Huang *et al.,* 2013) and *Terminalia arjuna* (Velide *et al.,* 2013). Our findings were in line with previous research indicating that non-infested or biocontrol-treated plants had greater photosynthetic pigment content required to sustain development under pest stress (Golawska *et al.,* 2010; Nagrare *et al.,* 2017). Overall, *T. harzianum* inoculation was associated with a significantly smaller loss of photosynthetic pigments under mealybug infestation; photosynthetic efficiency itself was not measured in this experiment.

Table 1. Morphological growth of tomato during biocontrol of *P. solenopsis* by *T. harzianum*.

Treatments	Morphological growth of tomato					
	PH (cm)	RD (cm)	PH/RD	SDW (g)	RDW (g)	SDW/RDW
Plant (control)	75.53	35.23	2.18	5.99	2.57	2.38
Plant + *P. solenopsis*	29.80	18.13	1.65	2.76	1.03	2.71
Plant + *P. solenopsis* + *T. harzianum*	52.67	31.17	1.70	4.80	1.96	2.45
Plant + *T. harzianum*	86.83	48.87	1.78	7.68	3.38	2.29
LSD at p≤0.05	7.39	5.88	NS (0.45)	0.56	0.52	NS (0.60)

PH: Plant height (cm); RD: Root depth (cm); SDW: Shoot dry weight (g); RDW: Root dry weight (g). Values are means of three replicates (n = 3). LSD: Least significant difference at p≤0.05 from one-way ANOVA (error df = 8); differences between two means larger than the LSD are significant. NS: Not significant (p>0.05)

Table 2. Photosynthetic pigments of tomato during biocontrol of *P. solenopsis* by *T. harzianum*.

Treatments	Photosynthetic pigments (mg/ g fresh weight)					
	Chlo. A	Chlo. B	A/B	A+B	Caroten.	Total Pig.
Plant (control)	1.19	0.49	2.43	1.68	0.28	1.96
Plant + *P. solenopsis*	0.75	0.29	2.54	1.04	0.16	1.20
Plant + *P. solenopsis* + *T. harzianum*	0.90	0.35	2.55	1.26	0.19	1.44
Plant + *T. harzianum*	1.31	0.52	2.52	1.82	0.31	2.14
LSD at p≤0.05	0.086	0.025	NS (0.350)	0.081	0.029	0.090

Chlo. A: Chlorophyll A; Chlo. B: Chlorophyll B; Caroten.: Carotenoids; Total pig: Total pigments. Values are means of three replicates (n = 3). LSD: Least significant difference at p≤0.05 from one-way ANOVA (error df = 8). NS: Not significant (p>0.05)

Table 3. Osmoprotectants compounds (Proline, µ mol/ g fresh weight; Glycine Betaine, µg / g fresh weight) and relative water content (%) of tomato during biocontrol of *P. solenopsis* by *T. harzianum*.

Treatments	Osmoprotectants compounds		Relative water content
	Proline	Glycine Betaine	
Plant (control)	20.41	3.64	89.98
Plant + *P. solenopsis*	70.65	13.43	50.34
Plant + *P. solenopsis* + *T. harzianum*	88.04	18.05	69.44
Plant + *T. harzianum*	32.31	4.96	92.21
LSD at p≤0.05	4.65	0.77	2.88

Values are means of three replicates (n = 3). LSD: Least significant difference at p≤0.05 from one-way ANOVA (error df = 8)

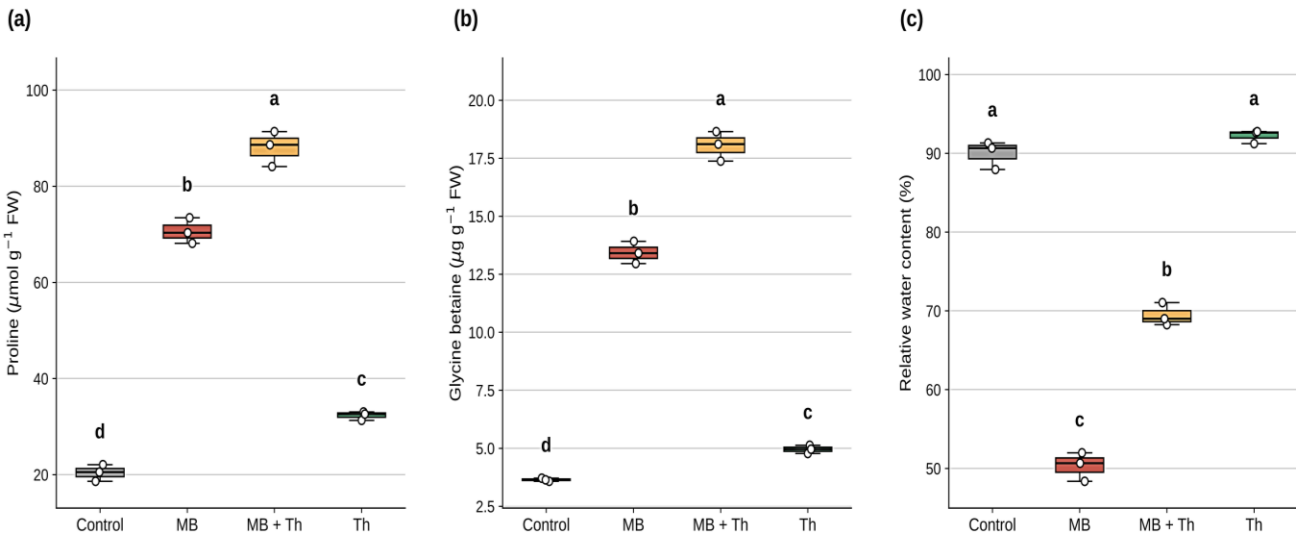


Fig. 6. Osmoprotectant accumulation and water status. Boxplots illustrating variations in (a) proline content, (b) glycine betaine (GB) content and (c) relative water content (RWC) under different treatments (Plant Control, Cotton Mealybug, Mealybug + *Trichoderma*, and *Trichoderma*). Each panel represents a separate physiological parameter. Individual points correspond to replicates (n = 3), while boxplots summarize data distribution and treatment effects. Different letters above the boxes indicate significant differences among treatments (the least significant difference (LSD) test, p≤0.05). MB, cotton mealybug; Th, *T. harzianum*.

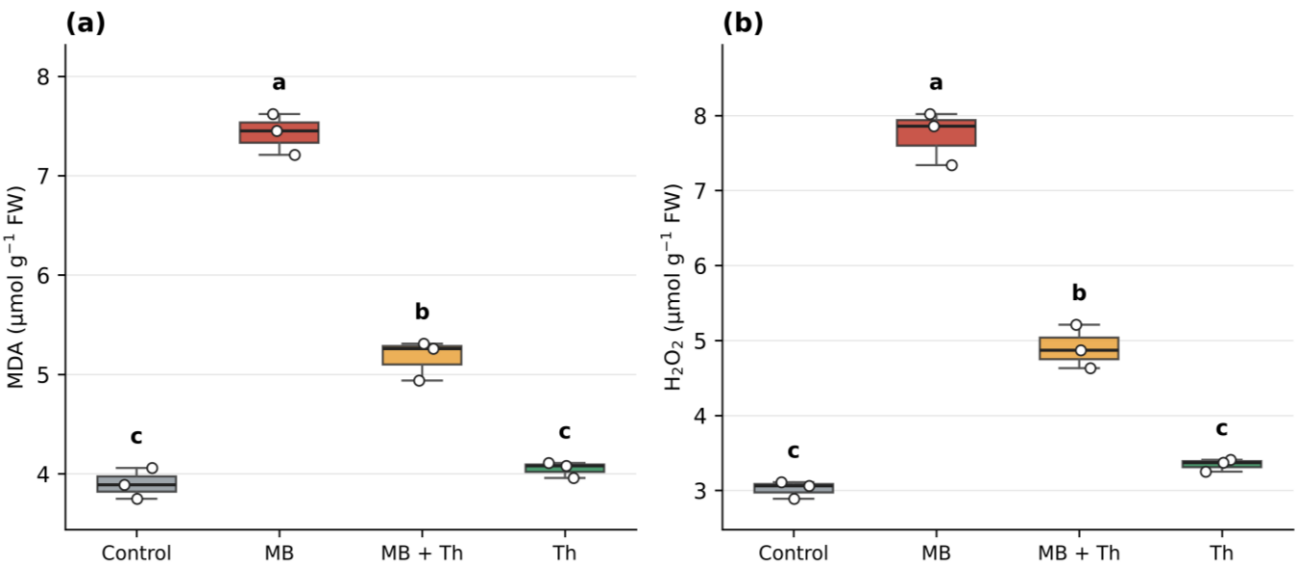


Fig. 7. Oxidative stress markers. Boxplots depicting the levels of (a) malondialdehyde (MDA) and (b) hydrogen peroxide (H_2O_2) under different treatments (Plant Control, Cotton Mealybug, Mealybug + *Trichoderma*, and *Trichoderma*). Each panel corresponds to a specific stress marker. Points represent replicates (n = 3), and boxplots summarize central tendency and variability, reflecting oxidative stress responses. Different letters above the boxes indicate significant differences among treatments (the least significant difference (LSD) test, p ≤ 0.05). MB, cotton mealybug; Th, *T. harzianum*.

Pigment loss under mealybug feeding is usually attributed to accelerated chlorophyll breakdown driven by reactive oxygen species, a reduced supply of nitrogen and magnesium to the leaves, and the shading effect of honeydew and sooty mould (Huang *et al.,* 2013; Huang & Zhang, 2016). The partial recovery of pigments in co-inoculated plants agreed with evidence that endophytic *Trichoderma* strains raised photosynthetic capacity by improving nutrient uptake, protecting chloroplasts from oxidative injury and upregulating photosynthesis-related genes (Harman *et al.,* 2021; Sood, 2026). Neither nutrient uptake nor the expression of photosynthesis-related genes were measured here, so these routes are offered as possible explanations rather than demonstrated mechanisms. The strong positive correlation between total pigments and the growth traits in the present study (r = 0.90 to 0.98; Fig. 9) is consistent with the view that maintenance of the photosynthetic apparatus is one of the routes through which *T. harzianum* reduces the growth penalty of infestation.

Effect of *Trichoderma* and cotton mealybug infestation on osmoprotectants: The osmoprotectants were substantially affected by various treatments (Table 3, Fig. 6). Control plants had low levels of proline (20.41 µmol g^{-1} FW) and glycine betaine (3.64 µg g^{-1} FW) and a high relative water content (RWC: 89.98%). Cotton mealybug infestation resulted in elevated levels of proline (70.65 µmol g^{-1} FW) and glycine betaine (13.43 µg g^{-1} FW), as

well as a significant decrease in RWC (50.34%), suggesting stress conditions. However, *Trichoderma* application in infested plants further increased osmoprotectants (proline: 88.04 µmol g^{-1} FW; GB: 18.05 µg g^{-1} FW) while improving RWC (69.44%) relative to infested plants. In contrast, *Trichoderma* alone maintained relatively lower osmoprotectant levels (proline: 32.31 µmol g^{-1} FW; GB: 4.96 µg g^{-1} FW) with the highest RWC (92.21%, not significantly different from the control), reflecting a favorable water status in the absence of infestation. The rise in proline and glycine betaine under infestation is consistent with reported increases in these solutes under insect attack (Mostafa *et al.,* 2022; Alvarez *et al.,* 2022), and comparable modulation of stress-related metabolites by beneficial microbes has been described previously (Zinan *et al.,* 2025). Higher osmolyte concentrations are not in themselves evidence of improved stress tolerance, however, because they may equally reflect the persistence of physiological stress; the osmolyte data are therefore interpreted below together with the water-status and oxidative-stress results rather than on their own.

Proline and glycine betaine are best known compatible solutes in drought and salinity, but they also accumulate during biotic stress, where proline scavenges hydroxyl radicals and singlet oxygen, stabilizes proteins and membranes, and feeds into redox and defense signalling (Alvarez *et al.,* 2022). In our study, the highest proline and glycine betaine levels occurred in co-inoculated plants, which at the same time held much more water than infested plants. This combination is consistent with an enhanced osmotic response under the joint effect of infestation and fungal inoculation. Two readings remain possible: the fungus may have amplified an adaptive osmotic adjustment that allowed leaf cells to retain water, or the high osmolyte concentrations may indicate that these plants were still experiencing substantial stress. Because osmotic adjustment was not measured directly including leaf water or osmotic potential, the present data cannot separate these alternatives, and the higher relative water content of co-inoculated plants relative to infested plants is the only direct evidence of better water status. *Trichoderma*-mediated increases in osmolyte accumulation, together with stronger antioxidant capacity, have likewise been reported under salinity in Indian mustard (Ahmad *et al.,* 2015) and *Ochradenus baccatus* (Hashem *et al.,* 2014). By contrast, the modest osmolyte levels in plants inoculated with *T. harzianum* alone show that the fungus did not impose a metabolic cost in the absence of stress. This pattern fits the concept of defense priming, in which beneficial microbes prepare the plant to respond faster and more strongly only after it is challenged (Pieterse *et al.,* 2014; Martínez-Medina *et al.,* 2017).

Effect of *Trichoderma* and cotton mealybug infestation on oxidative stress markers: Treatments had a significant impact on oxidative stress indicators such as MDA and H_2O_2 levels (Table 4, Fig. 7). Control plants had low levels of MDA (3.90 µmol g^{-1} FW) and H_2O_2 (3.02 µmol g^{-1} FW), suggesting a normal baseline redox status. In contrast, cotton mealybug infestation substantially raised MDA (7.43 µmol g^{-1} FW) and H_2O_2 (7.74 µmol g^{-1} FW), indicating severe oxidative damage. However, *T. harzianum* treatment of infested plants lowered these levels (MDA: 5.17 µmol g^{-1} FW; H_2O_2: 4.90 µmol g^{-1} FW), indicating the reduction of oxidative stress. Plants treated with *Trichoderma* alone maintained low MDA (4.05 µmol g^{-1} FW) and H_2O_2 (3.34 µmol g^{-1} FW), similar to control levels (not significantly different). Overall, *T. harzianum* inoculation was associated with significantly less oxidative damage in infested plants. These results are consistent with prior research indicating that biotic stress alters plant physiological condition, while beneficial microorganisms like *Beauveria bassiana* may modify oxidative responses and increase plant performance (Nchu *et al.,* 2022; Zinan *et al.,* 2025).

Relative to the control, infestation increased MDA by 90.4% and H_2O_2 by 156.3%, whereas the corresponding increases in co-inoculated plants were only 32.6% and 62.4%. The very close correlation between H_2O_2 and MDA ($r = 0.99$; Fig. 9) demonstrates that the excess H_2O_2 generated during feeding translated directly into lipid peroxidation of cell membranes. Reactive oxygen species produced at the feeding site are part of the early defense response and, at moderate levels, act as signals that switch on downstream defenses; when they accumulate for long periods, however, they damage membranes, proteins and chloroplasts (Mittler *et al.,* 2022). Because *P. solenopsis* can suppress effective JA-dependent defenses (Zhang *et al.,* 2015), infested plants may remain locked in a prolonged state of oxidative imbalance. The lower H_2O_2 and MDA contents of co-inoculated plants agree with reports that *Trichoderma* colonization strengthens antioxidant enzymes such as superoxide dismutase, catalase and ascorbate peroxidase, and thereby limits lipid peroxidation under stress (Ahmad *et al.,* 2015; Woo *et al.,* 2023). The antioxidant enzyme activities measured here (Section 3.6) are consistent with this interpretation, although an association between higher enzyme activity and lower H_2O_2 and MDA contents does not by itself establish the underlying mechanism. Endophytic *B. bassiana* has likewise been shown to modulate oxidative responses while improving the growth of *Fusarium*-infected tomato (Nchu *et al.,* 2022), which underlines that the final outcome depends on the balance between ROS signalling and ROS detoxification. Taken together with the proline response, our results are consistent with better control of cellular redox balance being an important component of the tolerance observed after *T. harzianum* inoculation.

Effect of *Trichoderma* and cotton mealybug infestation on oxidative enzymes (IAA Oxidase and PPO): Mealybug infestation significantly increased the activity of both oxidative enzymes (Table 5, Fig. 8). IAA oxidase rose from 0.411 U mg^{-1} protein in control plants to 0.597 U mg^{-1} protein in infested plants (+45.2%), and PPO from 0.153 to 0.369 U mg^{-1} protein (+141.5%). Co-inoculation with *T. harzianum* lowered IAA oxidase by 21.5% and PPO by 25.0% relative to infested plants, although both enzymes remained above control levels. In plants inoculated with *T. harzianum* alone, IAA oxidase did not differ from the control (0.405 U mg^{-1} protein), whereas PPO was moderately but significantly higher (0.194 U mg^{-1} protein).

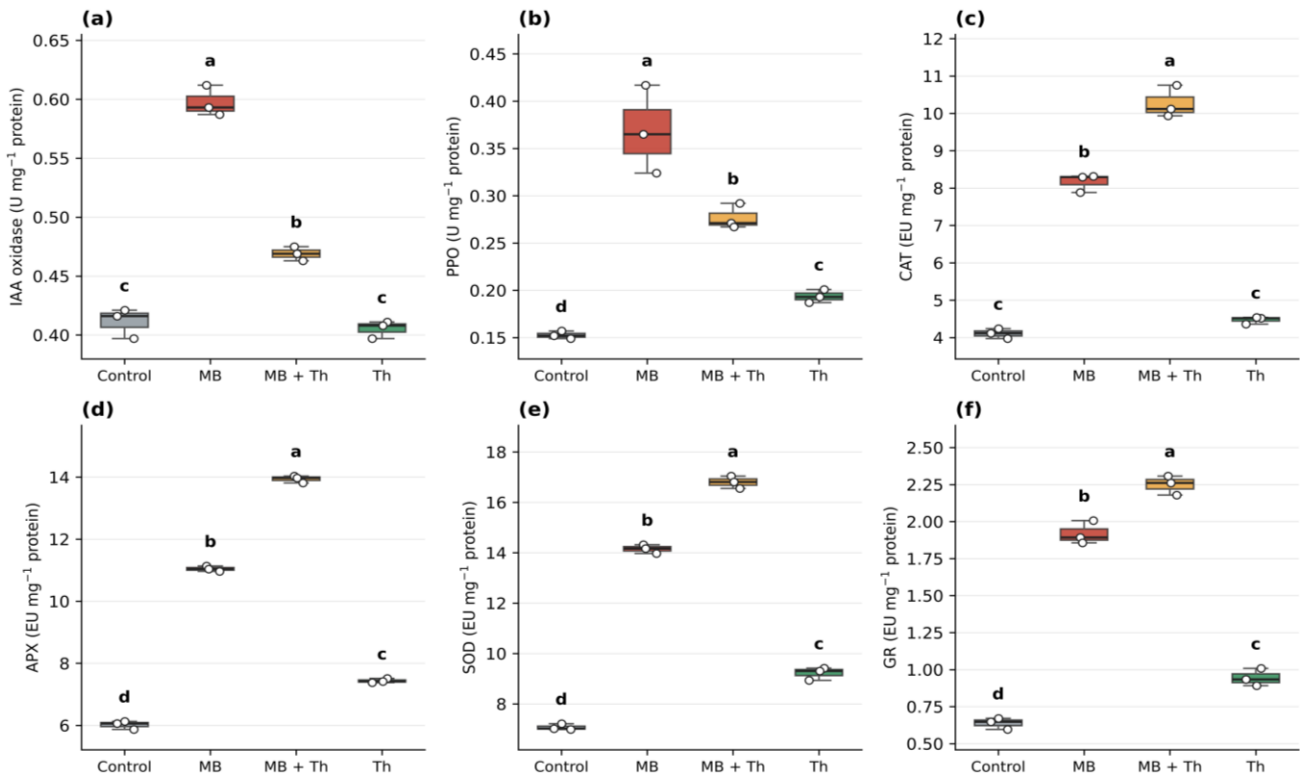


Fig. 8. Activities of oxidative and antioxidant enzymes in tomato under different treatments: (a) IAA oxidase, (b) polyphenol oxidase (PPO), (c) catalase (CAT), (d) ascorbate peroxidase (APX), (e) superoxide dismutase (SOD) and (f) glutathione reductase (GR). Boxplots show the median and interquartile range, and points are individual replicates (n = 3). Different letters indicate significant differences among treatments (the least significant difference (LSD) test, $p \leq 0.05$; PPO analysed after log10 transformation). MB, cotton mealybug; Th, *T. harzianum*.

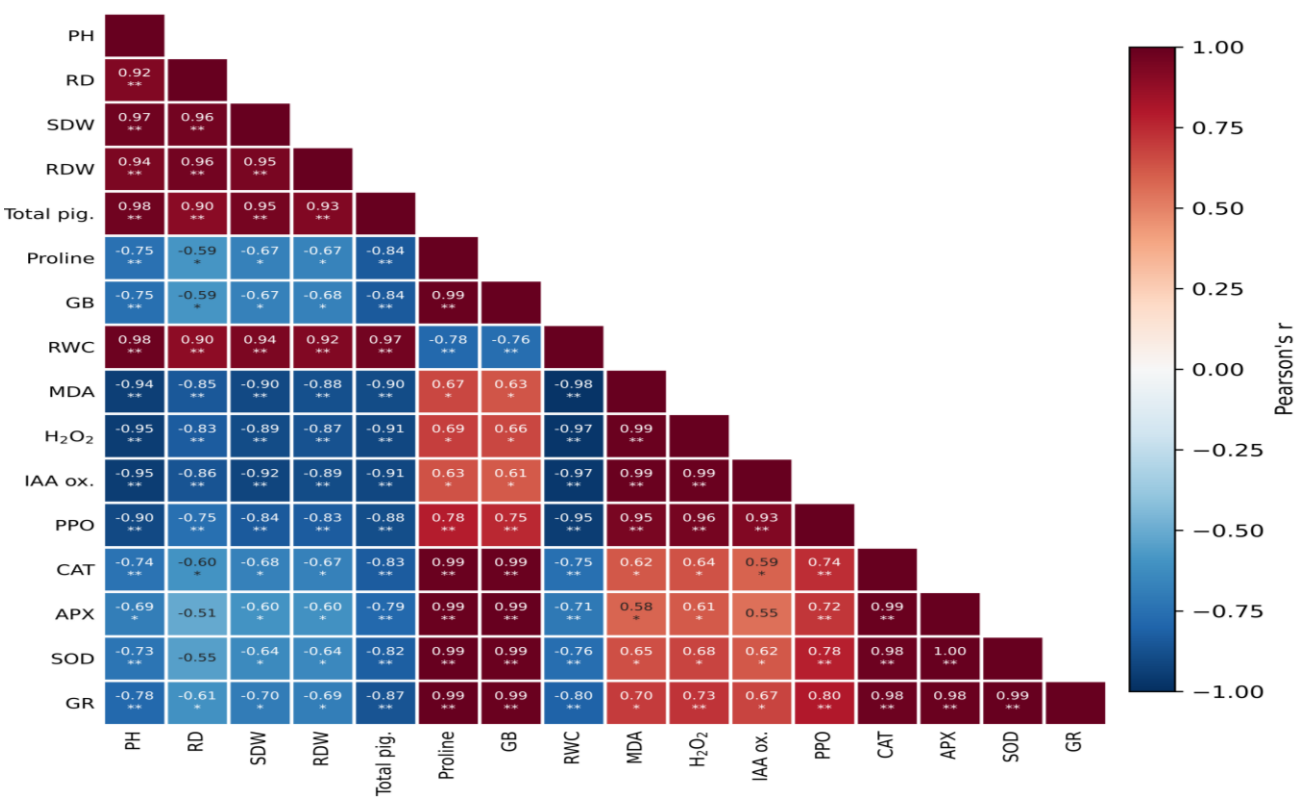


Fig. 9. Pearson correlation heatmap of growth, photosynthetic, osmotic, oxidative stress and enzyme traits of tomato (pooled data, n = 12). Colours show the correlation coefficient (r) from −1 (blue) to +1 (red); * and ** indicate significance at $p \leq 0.05$ and $p \leq 0.01$, respectively. PH, plant height; RD, root depth; SDW, shoot dry weight; RDW, root dry weight; Total pig., total pigments; GB, glycine betaine; RWC, relative water content; MDA, malondialdehyde; IAA ox., IAA oxidase; PPO, polyphenol oxidase; CAT, catalase; APX, ascorbate peroxidase; SOD, superoxide dismutase; GR, glutathione reductase.

Table 4. Oxidative stress markers H_2O_2 (µ mol/ g fresh weight) and MDA (µ mol/ g fresh weight) of tomato during biocontrol of *P. solenopsis* by *T. harzianum*.

Treatments	Oxidative stress markers	
	MDA	H_2O_2
Plant (control)	3.90	3.02
Plant + *P. solenopsis*	7.43	7.74
Plant + *P. solenopsis* + *T. harzianum*	5.17	4.90
Plant + *T. harzianum*	4.05	3.34
LSD at p≤0.05	0.32	0.45

H_2O_2: Hydrogen peroxide; MDA: Malondialdehyde. Values are means of three replicates (n = 3). LSD: Least significant difference at p≤0.05 from one-way ANOVA (error df = 8)

Table 5. Activities of the oxidative enzymes indole-3-acetic acid oxidase (IAA oxidase) and polyphenol oxidase (PPO) (U mg^{-1} protein) in tomato during biocontrol of *P. solenopsis* by *T. harzianum*.

Treatments	Oxidative enzymes	
	IAA oxidase	PPO
Plant (control)	0.411	0.153
Plant + *P. solenopsis*	0.597	0.369
Plant + *P. solenopsis* + *T. harzianum*	0.469	0.277
Plant + *T. harzianum*	0.405	0.194
LSD at p≤0.05	0.019	0.058†

IAA oxidase: Indole-3-acetic acid oxidase; PPO: Polyphenol oxidase. Values are means of three replicates (n = 3). LSD: Least significant difference at p≤0.05 from one-way ANOVA (error df = 8). †PPO was analysed after $\log_{10}$ transformation to satisfy the normality assumption; its LSD is therefore expressed on the $\log_{10}$ scale

IAA oxidase degrades indole-3-acetic acid, the main auxin controlling cell elongation and root development, so its strong induction in infested plants offers a plausible hormonal explanation for the marked reduction in plant height and root depth. In line with this, IAA oxidase was among the traits most negatively correlated with growth (r = −0.86 to −0.95; Fig. 9). *Trichoderma* species promote shoot growth and lateral root formation through auxin-dependent mechanisms (Contreras-Cornejo *et al.,* 2009), and restraining auxin catabolism, as observed in co-inoculated plants, would complement this effect. PPO oxidizes phenolic compounds to reactive quinones that bind dietary proteins and lower their nutritional value for herbivores, and work with tomato has shown that PPO directly limits the performance of insect pests (Bhonwong *et al.,* 2009). The strong PPO induction in infested plants therefore reflects an active defense response, although its close association with H_2O_2 and MDA (r = 0.96 and 0.95) indicates that it also tracks the intensity of tissue injury. The intermediate PPO activity of co-inoculated plants, and its modest rise in plants inoculated with *T. harzianum* alone, are consistent with a primed but less costly defensive state (Martínez-Medina *et al.,* 2017).

Effect of *Trichoderma* and cotton mealybug infestation on antioxidant enzymes: The antioxidant enzymes followed a different pattern (Table 6, Fig. 8). Compared with the control, infestation increased SOD, CAT, APX and GR activities by 100.1%, 98.7%, 83.5% and 200.6%, respectively. Co-inoculation raised these activities further, to the highest values recorded in the experiment (SOD 16.80, CAT 10.27, APX 13.94 and GR 2.25 U mg^{-1} protein), which were 18.7%, 25.8%, 26.2% and 17.2% higher than the infested plants. Plants inoculated with *T. harzianum* alone showed smaller but significant increases in SOD (30.4%), APX (23.5%) and GR (47.9%) over the control, whereas their CAT activity did not differ from the control.

SOD forms the first line of enzymatic defense by converting superoxide radicals into H_2O_2, which is then removed by CAT and by APX in the ascorbate–glutathione cycle, where GR regenerates reduced glutathione (Gill and Tuteja, 2010; Hasanuzzaman *et al.,* 2019). The coordinated rise of all 4 enzymes in co-inoculated plants therefore points to a greater capacity to remove H_2O_2 as soon as it is formed, which offers a likely explanation for the 36.6% lower H_2O_2 and 30.4% lower MDA contents of these plants compared with infested plants. Because insect numbers were not recorded, the two treatments cannot be assumed to have experienced identical pest pressure. Insect feeding is known to trigger ROS production and antioxidant responses that interact closely with photosynthesis and hormonal signalling (Kerchev *et al.,* 2012), and the profile of infested plants showed that these defenses were activated but not sufficient to prevent oxidative damage. *Trichoderma* inoculation has previously been shown to raise SOD, CAT, APX and GR activities and to reduce lipid peroxidation in plants exposed to salinity (Ahmad *et al.,* 2015) and to strengthen antioxidant defense in tomato under biotic stress of viral origin (Salami *et al.,* 2025); our results are consistent with a comparable response under insect-induced stress. The small but significant increases in plants inoculated with *T. harzianum* alone suggested that the endophyte maintained the antioxidant system in a primed state without a cost to growth, which was in fact greatest in this treatment. Endophytic colonization of the tissues was not quantified, so priming is inferred from the enzyme profile rather than demonstrated.

Table 6. Activities of the antioxidant enzymes catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and glutathione reductase (GR) (EU mg^{-1} protein) in tomato during biocontrol of *P. solenopsis* by *T. harzianum*.

Treatments	Antioxidant enzymes (EU mg^{-1} protein)			
	CAT	APX	SOD	GR
Plant (control)	4.11	6.02	7.07	0.64
Plant + *P. solenopsis*	8.16	11.04	14.15	1.92
Plant + *P. solenopsis* + *T. harzianum*	10.27	13.94	16.80	2.25
Plant + *T. harzianum*	4.47	7.43	9.22	0.94
LSD at p≤0.05	0.49	0.20	0.39	0.12

CAT: Catalase; APX: Ascorbate peroxidase; SOD: Superoxide dismutase; GR: Glutathione reductase; EU: Enzyme units. Values are means of three replicates (n = 3). LSD: Least significant difference at p≤0.05 from one-way ANOVA (error df = 8)

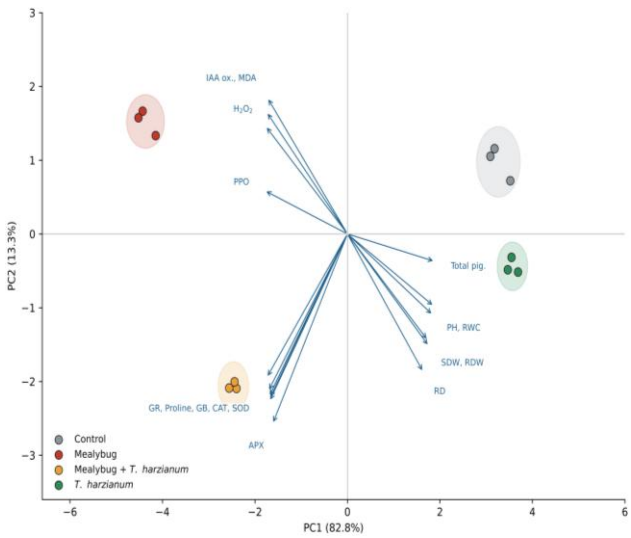


Fig. 10. Principal component analysis (PCA) biplot of all measured parameters, including enzyme activities. Points are individual replicates coloured by treatment, shaded ellipses group the replicates of each treatment, and arrows show the variable loadings. PC1 and PC2 explain 82.8% and 13.3% of the total variance, respectively.

ANOVA of treatment effects on plant attributes: Treatment effects were highly significant ($p<0.001$) for all measured parameters, with F values (df = 3, 8) ranging from 39.1 for root dry weight to 3508.4 for ascorbate peroxidase. The only exceptions were the PH/RD, SDW/RDW and chlorophyll a/b ratios, which did not differ among treatments ($p>0.05$). The ranking of the treatments for each individual trait is given in Tables 1 to 6 and Figures 4 to 8 and is described in Sections 3.1 to 3.6; it is not repeated here.

Treatment effects on plant traits: boxplot with LSD: Boxplot analysis, along with the LSD test, revealed significant differences between treatments for all morphophysiological and biochemical parameters. Cotton mealybug infestation decreased growth parameters (plant height, root depth, shoot and root dry weight), photosynthetic pigments, and relative water content, but increased osmoprotectants (proline) and oxidative stress indicators (MDA and H_2O_2). *Trichoderma*-treated plants, on the other hand, consistently demonstrated greater growth performance, increased pigment content, and improved hydration status, distinguishing them from other statistical groups. It should be noted, however, that RWC, MDA and H_2O_2 of plants inoculated with Trichoderma alone did not differ significantly from the control under the LSD test, whereas their growth traits and photosynthetic pigments were significantly higher. The combined treatment (mealybug + *Trichoderma*) elicited intermediate responses, suggesting that stress effects were partially mitigated; for root depth the recovery was complete, as this trait did not differ from the control, whereas root dry weight remained significantly lower. For the enzymes (Fig. 8), all four treatments formed distinct groups for APX, SOD, GR and PPO, while IAA oxidase and CAT activities of plants inoculated with *Trichoderma* alone did not differ from the control. In summary, the LSD groupings showed that inoculation with *T. harzianum* was associated with a significant reduction in mealybug-induced physiological stress and with better plant performance (Figs. 4 to 8).

Correlation analysis of plant growth, physiological, and stress parameters: The Pearson correlation analysis revealed strong and statistically significant associations among plant growth, physiological, and stress indicators (Fig. 9). Plant height (PH), root depth (RD), shoot dry weight (SDW), and root dry weight (RDW) had a positive relationship ($r = 0.92$ to 0.98, $p<0.01$) with each other and with total chlorophyll content, suggesting that healthier, taller plants retained better photosynthetic capability. Proline and glycine betaine, osmoprotectants, showed a positive correlation ($r = 0.99$, $p<0.01$) but a negative correlation with relative water content (RWC; $r = -0.76$ to -0.78) and growth metrics ($r = -0.59$ to -0.75, $p<0.05$), indicating osmotic stress during mealybug infestation. Stress indicators, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), were highly positively correlated ($r = 0.99$, $p<0.01$) and inversely correlated to growth, pigments, and RWC ($r = -0.83$ to -0.98), demonstrating the detrimental effects of oxidative stress. Because the coefficients were calculated on pooled data from all treatments (n = 12), they mainly reflect treatment-driven variation. They therefore describe how the traits responded jointly to the treatments and cannot be used to infer causal links between individual traits. They show that the traits favoured by *Trichoderma* (growth, pigments and RWC) are precisely those depressed by oxidative stress, which is consistent with, although it does not prove, a central role for restricted ROS accumulation in the response to the fungus (Fig. 9). When the enzymes were included, IAA oxidase and PPO were strongly and negatively correlated with growth traits ($r = -0.75$ to -0.95) and RWC ($r = -0.95$ to -0.97), and positively correlated with H_2O_2 and MDA ($r = 0.95$ to 0.99). The four antioxidant enzymes were almost perfectly intercorrelated ($r = 0.98$ to 1.00) and closely followed proline and glycine betaine ($r = 0.99$), but were only moderately related to H_2O_2 and MDA ($r = 0.58$ to 0.73). This is the pattern expected when antioxidant capacity rises most in the treatment where oxidative damage is partly contained, namely co-inoculation (Fig. 9).

Principal component analysis (PCA) of all parameters: The PCA, which included all assessed traits, including plant growth characteristics, photosynthetic pigments, osmoprotectants, stress indicators, and oxidative and antioxidant enzymes, indicated clear treatment clusters (Fig. 10). The first two principal components accounted for 82.8% and 13.3% of the overall variance, respectively, and together accounted for 96.1% of it. The *Trichoderma*-only and control treatments were located on the same side of PC1, associated with high growth, pigment and RWC values and low stress indicators, with *Trichoderma*-treated plants positioned furthest along this axis. The Mealybug + *Trichoderma* treatment occupied an intermediate position along PC1 and was separated from all other treatments along PC2, which was driven mainly by proline and glycine betaine together with the four antioxidant enzymes; this reflects its distinctive combination of partly restored growth and the highest osmoprotectant accumulation and antioxidant enzyme activity. The mealybug-only treatment occupied a distinct position along PC1, with lower growth, photosynthetic pigments, and relative water content, as well as elevated stress signal levels. Growth parameters (PH, RD, SDW, RDW), photosynthetic pigments and RWC loaded on one side of PC1, whereas MDA, H_2O_2 and the osmoprotectants, IAA oxidase, PPO and the antioxidant

enzymes loaded on the opposite side, so PC1 can be read as a gradient from healthy to stressed plants. Because the analysis is a descriptive ordination of standardized trait values from twelve observations, it summarizes the separation of the treatments but does not test hypotheses about the processes responsible for that separation.

Integrated interpretation, practical implications and limitations: Taken together, the univariate and multivariate results are compatible with several non-exclusive explanations for the lower physiological cost of infestation in co-inoculated plants. These are set out as working hypotheses, because none of the underlying processes was measured directly in the present experiment. First, the better root development of co-inoculated plants may have improved water uptake, which would be consistent with their higher relative water content, and may have helped maintain the supply of nutrients needed for chlorophyll synthesis; neither water nor nutrient uptake was measured. Second, co-inoculated plants accumulated the most proline and glycine betaine while holding more water than infested plants, a pattern consistent with an enhanced osmotic response, although the accumulation of these solutes may equally reflect continuing stress. Third, the higher activities of SOD, CAT, APX and GR in these plants are associated with a smaller build-up of H_2O_2 and with less lipid peroxidation are likely to protect membranes and the photosynthetic apparatus. Fourth, the smaller rise in IAA oxidase activity in co-inoculated plants may have limited auxin catabolism and so helped preserve the auxin pool needed for growth, while PPO-mediated defense remained partly active; indole-3-acetic acid concentrations themselves were not quantified. These effects are consistent with the priming of JA-regulated defenses and ISR by *Trichoderma* (Pieterse *et al.,* 2014; Martínez-Medina *et al.,* 2017; Coppola *et al.,* 2019), which may counteract the suppression of JA signalling that *P. solenopsis* uses to its advantage (Zhang *et al.,* 2015). Defense hormones and the expression of defense genes were not measured here, and endophytic colonization of tomato tissues was not confirmed, so the involvement of jasmonate-dependent priming and induced systemic resistance in the responses reported above remains to be tested. From a practical standpoint, an endophyte that improves host tolerance could in principle complement the parasitoids and predators that already regulate mealybug populations in the field (Arif *et al.,* 2012; El Aalaoui *et al.,* 2024, 2025; Namadara *et al.,* 2025), and jasmonate-dependent defense induction in tomato has been reported not to reduce the density of predatory biological control agents (Bohloolzadeh *et al.,* 2025). Compatibility with natural enemies was not tested in the present study, however, and any contribution of *T. harzianum* to the integrated management of *P. solenopsis* should be regarded as a research prospect that still requires direct measurement of pest suppression under field conditions.

Several limitations should be considered when interpreting these results. The experiment was carried out with a single cultivar under growth-chamber conditions and with three biological replicates per treatment, which limits the precision of the variance estimates and the power to detect small differences; the results therefore require confirmation in larger and independently repeated experiments. Mealybug population size, survival and fecundity were not recorded, so the present data describe the plant's tolerance to infestation rather than direct suppression of the pest. Endophytic colonization of tomato tissues by *T. harzianum* was not quantified at harvest, and defense hormones and the expression of defense genes were not measured. Future work combining insect performance assays, re-isolation of the endophyte, hormone profiling, gene expression analysis, and multi-season field trials would clarify how far the benefits observed here can be translated into practical mealybug management.

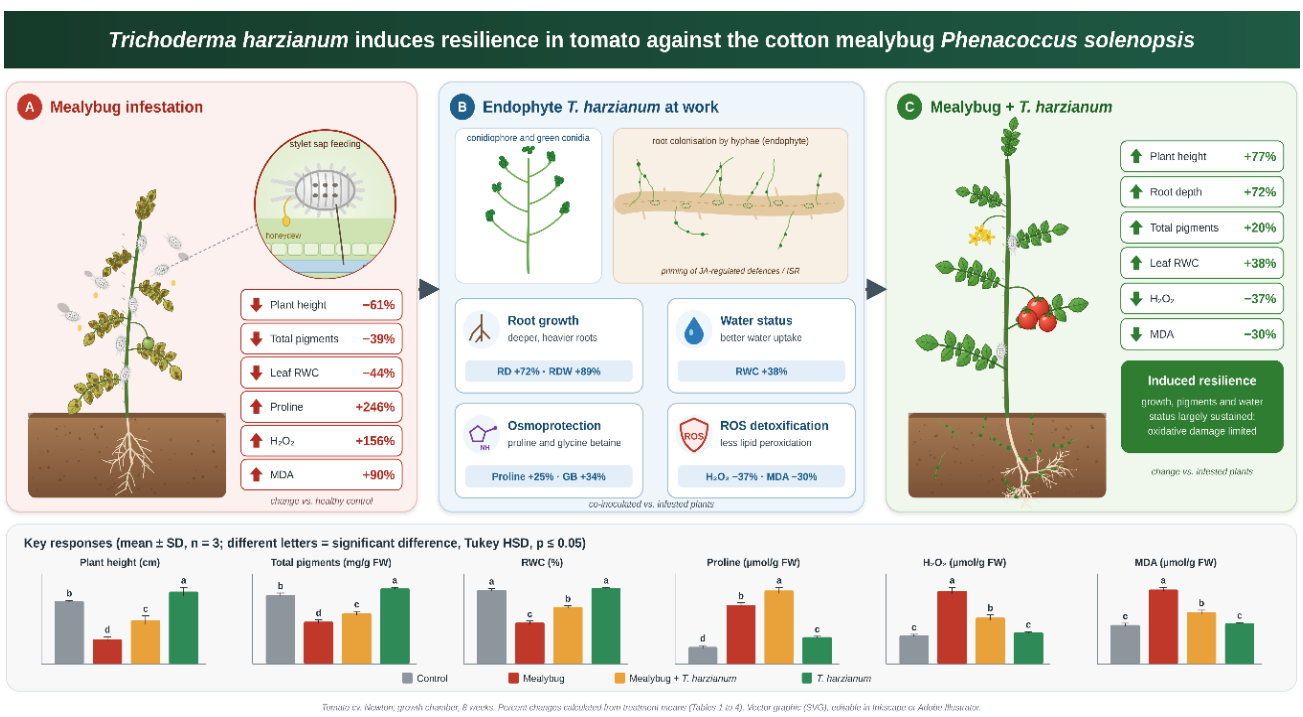


Fig. 11. Graphical abstract described the efficiency of biocontrol agent *Trichoderma harzianum* to induce resilience in tomato (*Lycopersicon esculentum* cv. Newton) against cotton mealybug, (*Phenacoccus solenopsis*).

Conclusion

The research indicates that *P. solenopsis* infestation impairs tomato growth, photosynthetic efficiency and hydration status, and induces oxidative stress. Inoculation with the fungal endophyte *T. harzianum* mitigated these effects, with higher growth, chlorophyll content and relative water content, and less oxidative damage, than in infested plants. These benefits were associated with higher activities of SOD, CAT, APX and GR and with a smaller rise in IAA oxidase and PPO activities in infested plants. Combining *T. harzianum* inoculation with mealybug infestation partly restored plant performance, which is consistent with a role in stress mitigation. Univariate (ANOVA with LSD) and multivariate (correlation and PCA) analyses revealed significant differences among treatments in the morphophysiological and biochemical variables measured. On the evidence presented here, therefore, *T. harzianum* strengthens the plant's own tolerance mechanisms. Its capacity for direct suppression of *P. solenopsis* was not measured, it still needs to be quantified for this fungus-pest combination before entomopathogenic endophytes can be recommended as a component of integrated mealybug management in tomato (Fig. 11).

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