

Enhancing natural disease resistance in greenhouse tomato through salicylic acid and sucrose-induced antioxidant defense against Fusarium wilt

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ABSTRACT

Fusarium wilt, caused by *Fusarium oxysporum*, is one of the most destructive soil-borne diseases affecting tomato (*Solanum lycopersicum* L.) production worldwide, including in Algeria, where it represents a major phytosanitary constraint. Given the limitations of conventional chemical control, this study investigated the individual and combined effects of salicylic acid (SA) and sucrose (S), applied at increasing concentrations (0.4, 0.5, and 0.6 mM), as eco-friendly elicitors of induced resistance against *Fusarium oxysporum* in tomato. Fifty-three fungal isolates were recovered from wilted tomato plants collected across three Algerian regions, with the most virulent isolate molecularly identified as *Fusarium oxysporum* with 100% ITS sequence similarity; GenBank accession PZ939396. Disease severity, photosynthetic pigments, oxidative stress markers (H₂O₂, MDA), proline content, and antioxidant enzyme activities (SOD, CAT) were evaluated under greenhouse conditions. *Fusarium oxysporum* inoculation caused severe disease symptoms (92% disease index), marked pigment loss, and pronounced oxidative damage. Both elicitors reduced disease severity and oxidative stress in a dose-dependent manner, with SA consistently outperforming sucrose. The combined treatment (SA+S) provided the strongest protection, achieving the lowest disease index (12%; 86.96% protection), near-complete restoration of chlorophyll content, and the highest antioxidant enzyme activities and proline accumulation among all treatments. Multivariate analyses (PCA, HCA, Pearson correlation) showed a clear discrimination between infected and elicitor-treated plants, consistent with a coordinated pattern of antioxidant and osmoprotective responses. These findings indicate that the combined treatment (SA+S) provides greater protection than either elicitor applied alone, an effect not previously characterized against *Fusarium oxysporum*. This combined benefit, evidenced through an integrated physiological and biochemical approach in a locally relevant Algerian pathosystem, supports the potential integration of the combined SA and sucrose treatment into sustainable Fusarium wilt management strategies.

Keywords: *Solanum lycopersicum*, *Fusarium oxysporum*, salicylic acid, sucrose signaling, antioxidant enzymes.

INTRODUCTION

Solanum lycopersicum L. (tomato) is a member of the Solanaceae family, which comprises more

than 3000 economically important species, including potato, eggplant, tobacco, and pepper (*Capsicum*). Within this family, the genus *Solanum* is the largest, encompassing approximately 1250–1700

species distributed across most temperate and tropical regions of the world (Brambilla et al., 2022; González et al., 2011; S.K. Singh et al., 2023; V.K. Singh et al., 2018; Wakil et al., 2018).

Tomato is one of the world's most economically important vegetable crops and among the most extensively traded vegetables internationally. Together with onion and pepper, it accounts for nearly 63% of global vegetable production, highlighting its major contribution to global food systems and agricultural economies (Cao et al., 2026). Global tomato production exceeds 180 million tonnes annually, providing substantial food supplies and income, particularly in developing countries. The crop is cultivated on approximately 5 million hectares worldwide for both fresh consumption and industrial processing, with an average per capita consumption of nearly 20 kg (Abate, 2026). Its wide range of uses, from fresh consumption to processed products such as sauces and juices, further contributes to its considerable market value (Arah et al., 2015). In 2023, tomatoes destined for processing alone accounted for more than 45 million tonnes, representing nearly one-quarter of total global tomato production (Abate, 2026).

In North Africa and the Mediterranean Basin, tomato cultivation plays an important role in agricultural production and regional economies, with the crop being widely cultivated throughout the Mediterranean region (Panno et al., 2021). In Algeria, tomato production is affected by various phytopathological constraints, including soil-borne fungal pathogens that can significantly compromise plant growth and yield (Debbi et al., 2018). Among these pathogens, *Fusarium oxysporum*, the causal agent of Fusarium wilt, is one of the major soil-borne pathogens affecting tomato and is widely distributed throughout the Mediterranean basin (Panno et al., 2021). In Algeria, the occurrence of *Fusarium oxysporum* has been documented in several tomato-growing areas, highlighting its importance as a significant constraint to tomato production in the country (Debbi et al., 2018). The pathogen infects plants through the root system, colonizes the vascular tissues, and progressively induces chlorosis, wilting, and eventually plant death (Michielse and Rep, 2009). As colonization advances, *Fusarium oxysporum* spreads through the xylem, causing characteristic dark brown vascular discoloration that extends toward the stem apex, ultimately resulting in vascular dysfunction, irreversible wilting, and plant

collapse (Srinivas et al., 2019). Moreover, the production of long-lived chlamydospores enables the pathogen to survive in soil for many years in the absence of a host, making its eradication particularly difficult once fields become infested (Agrios, 2008). These characteristics, together with the soil-borne nature and persistence of the pathogen, make Fusarium wilt particularly difficult to manage and contribute to the recurrent occurrence of the disease in tomato-growing areas.

Management of Fusarium wilt traditionally relies on an integrated disease management (IDM) approach combining resistant cultivars, crop rotation, soil sanitation, and the judicious use of chemical fungicides. Additional cultural practices, including the use of certified disease-free seeds or transplants, disinfection of agricultural tools, sanitation of irrigation systems, and the application of organic amendments or biological control agents, contribute to reducing disease incidence and limiting pathogen spread (Jones et al., 2016; Srinivas et al., 2019). The long-term effectiveness of these strategies remains limited. Intensive reliance on chemical fungicides has raised concerns regarding environmental contamination, adverse effects on non-target organisms, and the emergence of fungicide-resistant pathogen populations (López-Zapata et al., 2021). The durability of genetic resistance is frequently undermined by the evolution of new virulent races capable of overcoming resistance genes, highlighting the need for sustainable integrated management strategies (Srinivas et al., 2019; Swett et al., 2023).

These limitations have stimulated growing interest in environmentally sustainable alternatives based on the activation of the plant's innate defense mechanisms. Rather than acting directly on the pathogen, elicitor-based approaches enhance the plant's biochemical and molecular defense responses and are increasingly regarded as a promising component of integrated and sustainable strategies for the management of Fusarium wilt in tomato (Haruna S. G. et al., 2024; Heikal et al., 2025).

SA is recognized as one of the principal regulators of plant immunity, notably through its role in the establishment of systemic acquired resistance (SAR), which is associated with the expression of pathogenesis-related (PR) genes (Palmer et al., 2017; Zavaliev and Dong, 2024).

SA also contributes to the maintenance of redox homeostasis by stimulating antioxidant enzymes, thereby limiting oxidative damage while supporting immune responses (Decsi et al., 2025).

Sucrose, long regarded as a simple energy source, is now recognized as a signaling molecule involved in the regulation of plant immunity (Eom et al., 2024; Morkunas and Ratajczak, 2014; Moustafa-Farag et al., 2020). Its exogenous application promotes the expression of defense genes and SA biosynthesis, enhancing resistance to bacterial and fungal pathogens (Yamada and Mine, 2024).

The individual protective effects of SA against *Fusarium* wilt in tomato have been well documented, particularly through improved SOD and CAT activities and the reduction of oxidative stress markers (H_2O_2 , MDA) in *Fusarium oxysporum*-infected plants (AL-surhane, 2022; Heikal et al., 2025). Similarly, sucrose-mediated sugar signaling has been associated with enhanced antioxidant capacity and the activation of systemic resistance against *Fusarium oxysporum* (Morkunas and Ratajczak, 2014; Xu et al., 2025; Yamada and Mine, 2024). However, the combined effect of SA and sucrose as a dual elicitor strategy against *Fusarium oxysporum* in tomato remains, to our knowledge, largely unexplored, as most previous studies have focused on SA alone or in combination with bio-control agents (AL-surhane, 2022; Ansari et al., 2025; Mufti et al., 2023; Zehra et al., 2017), while sucrose has mainly been studied in the context of abiotic stress (Sevgi and Leblebici, 2025; Wang et al., 2026). The potential combination of sugar signaling and SA-induced SAR therefore remains unexplored in the tomato–*Fusarium oxysporum* pathosystem, particularly under Algerian agroecological conditions, where *Fusarium* wilt constitutes a major phytosanitary constraint.

This study aims to establish innovative and sustainable methods for managing *Fusarium* wilt in tomato plants, prioritizing environmentally friendly and health-promoting solutions. It emphasizes the protective potential of SA and sucrose, applied individually at increasing doses or in combination, as eco-safe elicitors against *Fusarium oxysporum*. Additionally, the research incorporates biochemical analyses alongside experimental evaluations to investigate the modulation of antioxidant enzyme activities (SOD and CAT), photosynthetic pigment preservation, oxidative stress markers (H_2O_2 and MDA), and proline-mediated osmotic adjustment induced by these elicitors in infected *Solanum lycopersicum* plants. By comparing their individual and combined effects, this study clarifies the added protective value of the combined SA and sucrose treatment relative

to each elicitor alone, and provides an integrated physiological and biochemical framework for evaluating eco-friendly elicitor combinations against *Fusarium* wilt.

MATERIAL AND METHODS

Site of experimental

The experiment was conducted under controlled greenhouse conditions at the Department of Agronomy, University of Mohamed Khider Biskra, located in the wilaya of Biskra, northeastern Algeria (34.84° N, 5.73° E).

Isolation and identification of *Fusarium oxysporum*

Tomato plants exhibiting typical *Fusarium* wilt symptoms were collected from three major tomato-producing regions in Algeria (Ain Nagga, Leghrous, and Tolga). Stem tissues (approximately 5 mm) were surface-sterilized in 5% sodium hypochlorite for 2 min, rinsed four times with sterile distilled water, air-dried on sterile filter paper, and aseptically placed onto potato dextrose agar (PDA) medium supplemented with streptomycin (0.16 g L^{-1}) to suppress bacterial contamination. PDA was prepared according to the manufacturer's instructions and sterilized by autoclaving at 121 °C for 15 min. Plates were incubated at room temperature for 3 days, after which emerging colonies were subcultured on fresh PDA to obtain pure isolates. Fungal identification was based on colony morphology and microscopic characteristics using standard taxonomic keys (Agrios, 2008; Vi, 2017; Vi and Ra, 2016).

Virulence assay

The virulence of the *Fusarium* isolates was evaluated under greenhouse conditions at the Department of Agronomy, University Mohamed Khider of Biskra. Tomato seedlings at the three-true-leaf stage were inoculated with conidial suspensions (10^6 spores mL^{-1}) of three isolates (FO1, FO2, and FO3), while non-inoculated plants served as controls. Each treatment consisted of three biological replicates. Disease symptoms, including leaf yellowing, wilting, and vascular discoloration, were monitored throughout the experiment. Disease severity was assessed by

comparing inoculated plants with the control, and the isolate producing the highest disease severity was selected for subsequent experiments.

Molecular characterization

Three fungal isolates obtained from infected tomato plants were initially evaluated for their pathogenicity through a virulence test. Based on the results, the most virulent isolate was selected for molecular identification and used for further analysis in this study. Molecular characterization was performed by amplifying the internal transcribed spacer region (ITS1–5.8S–ITS2) of ribosomal DNA (rDNA) using polymerase chain reaction (PCR), followed by sequencing of the amplified products.

The ITS region was amplified using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR reaction mixture (total volume of 20 μ L) contained 4 μ L of 5 $\times$ reaction buffer, 1.6 μ L of $MgCl_2$ (25 mM), 0.3 μ L of dNTP mix (10 mM), 0.3 μ L of each primer (10 μ M), 1 unit of GoTaq® DNA polymerase (Promega, Madison, WI, USA), and 1 μ L of genomic DNA. Amplification was conducted using a VERITI thermocycler (Applied Biosystems, Foster City, CA, USA) with an initial denaturation at 94 °C for 3 minutes, followed by 35 cycles consisting of denaturation at 94 °C for 30 seconds, annealing at 58 °C for 45 seconds, and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 7 minutes.

The amplified products (10 μ L) were separated on a 1.5% (w/v) agarose gel prepared in TAE buffer to confirm successful amplification, with the expected size for the target molecular marker 650 bp. The PCR products were then purified and sequenced in both directions using the ITS primer pair (ITS1 and ITS4). Species identification was achieved by comparing the obtained sequences with those available in the National Center for Biotechnology Information (NCBI) database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), based on the highest sequence similarity.

Experimental design and foliar application of elicitor treatments

A pot experiment was conducted under semi-controlled greenhouse conditions at the Department of Agronomy, University of Mohamed Khider, to evaluate the effects of SA and sucrose on

the resistance of a commercial tomato (*Solanum lycopersicum* L.) variety, widely cultivated in the region, against *Fusarium oxysporum*. Uniform 35-day-old tomato seedlings were transplanted into 2.5 L pots containing sterilized, commercial peat-based growing substrate (BVB Substrates, Belgium), composed of approximately 90% black peat and 10–40% blonde peat, amended with PG-Mix fertilizer (NPK 12-14-24 + trace elements, 1.2–1.7 kg/m³), with a pH adjusted to 5.5–6.0 and characterized by high water retention combined with adequate air porosity for root development. Each treatment consisted of five biological replicates, with each replicate corresponding to a single plant grown in an individual pot ($n = 5$ plants per treatment). The plants were maintained under semi-controlled greenhouse conditions, with a mean temperature of approximately 30 °C, a relative humidity of approximately 60–70%, and a natural photoperiod of 13 h 24 min light/10 h 36 min darkness.

The treatments included a non-inoculated control (C), an inoculated untreated control (IF), sucrose at 0.4, 0.5, and 0.6 mM (S1, S2, and S3), salicylic acid at 0.4, 0.5, and 0.6 mM (SA1, SA2, and SA3), and a combined treatment of 0.6 mM SA + 0.6 mM sucrose (SA+S). Elicitor solutions were freshly prepared from analytical-grade reagents following previously published protocols (Arya et al., 2022; Mandal et al., 2009).

Treatments were applied to both leaves and roots once daily for seven consecutive days. Foliar applications were performed in the early morning (07:00–09:00 h) using a manual sprayer (approximately 20 mL plant⁻¹) to ensure uniform leaf coverage. Root applications were carried out by the soil drench method, whereby approximately 20 mL of the respective elicitor solution was poured directly onto the substrate around the base of each plant, allowing gradual absorption by the root system. Soil drench treatments were applied at the same time each day as the foliar sprays to maintain consistency between the two application routes. Pathogen inoculation (10⁶ conidia mL⁻¹) was carried out 48 h after the final elicitor application to allow activation of plant defense responses (Ansari et al., 2025; Zehra et al., 2017). Root infection was induced using the soil drench inoculation method. This method involved pouring 20 mL of *Fusarium oxysporum* spore suspension (10⁶ spores mL⁻¹) uniformly over the soil surface. The spore suspension infiltrated through the potting substrate, allowing direct contact between

the pathogen conidia and the plant roots, thereby establishing infection at the root level (Lai et al., 2020). Control plants received the same volume of sterile distilled water.

Disease index and induced protection

The disease symptoms were assessed on 56-day-old plants, and the disease index and percentage of induced protection were evaluated according to (AL-surhane, 2022).

Symptomatic plants were identified through systematic visual observation and physical counting at each assessment time point. Disease severity was rated on a scale of 1 to 5, adapted from (De Cal et al., 1995) with the addition of an intermediate level to account for early symptom onset, as follows: 0 — plant completely healthy, all leaves green, no visible symptoms; 1 — initial/slight yellowing restricted to the lowest leaf or leaves; 2 — yellowing of lower leaves; 3 — necrosis and death of lower leaves; 4 — wilting of upper leaves accompanied by complete death of lower leaves; 5 — complete plant death. This rating scale is consistent with similar approaches used in other pathosystems (Bindal et al., 2023).

Pathogen re-isolation following inoculation

The pathogen was re-isolated from symptomatic tissues following inoculation (Nirmaladevi et al., 2016). At 21 days post-inoculation, stem tissues were surface-sterilized in 5% sodium hypochlorite for 2 min, rinsed four times with sterile distilled water, air-dried on sterile filter paper, and aseptically placed onto potato dextrose agar (PDA) medium supplemented with streptomycin (0.16 g L^{-1}) to suppress bacterial contamination. Plates were incubated at $25 \pm 2 \text{ }^{\circ}\text{C}$ for 7 days. Fungal identification was based on microscopic characteristics using standard taxonomic keys, and re-isolated strains were compared with the original inoculum to confirm identity (Dihazi et al., 2025).

Biochemical and physiological parameters measurements

Quantification of photosynthetic pigments

The contents of chlorophylls were determined using 0.3 g of fresh leaf material, which was homogenized in cold 80% acetone. After extraction, the homogenate was centrifuged and the

absorbance of the clear supernatant was recorded at 663 nm and 645 nm.

$$\text{Chlorophyll } a = [12.7 \times A_{663}] - (2.69 \times A_{645}) \quad (1)$$

$$\text{Chlorophyll } b = [22.9 \times A_{645}] - (4.68 \times A_{663}) \quad (2)$$

The total carotenoid content (TCC) was determined as follows. Briefly, 500 mg of dry plant material was extracted with 15 mL of hexane. The mixture was vortexed thoroughly and allowed to stand for several minutes before centrifugation at 3000 rpm for 1 min. The supernatant was collected, and the extraction procedure was repeated until the plant material became colorless. The combined supernatants were evaporated to dryness at $40 \text{ }^{\circ}\text{C}$ using a rotary evaporator. The dried extract was then redissolved in 5 mL of hexane, and the absorbance was measured at 450 nm. The total carotenoid content was expressed as mg g^{-1} of extract based on the absorbance measured at 450 nm (Nobossé et al., 2018).

Hydrogen peroxide, malondialdehyde (MDA) and proline content

Hydrogen peroxide (H_2O_2) content was determined according to the method described by (Junglee et al., 2014) with the experimental procedure specified as follows. Fresh leaf tissue (0.20 g) was homogenized in 2 mL of 1% (w/v) trichloroacetic acid (TCA) using a mortar and pestle maintained on ice. The homogenate was centrifuged at 1000 rpm for 15 min at $4 \text{ }^{\circ}\text{C}$, and the resulting supernatant was collected. For the determination, 500 μL of the supernatant was mixed with 500 μL of 10 mM sodium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide (KI). The reaction mixture was incubated for 15 min in the dark, and the absorbance was subsequently measured at 390 nm using a spectrophotometer. H_2O_2 concentration was determined from a calibration curve prepared using known concentrations of H_2O_2 and expressed as $\mu\text{mol g}^{-1}$ fresh weight (FW).

Lipid peroxidation was evaluated by determining the malondialdehyde (MDA) content in leaf tissues following the method described by (Chen and Zhang, 2016), with the experimental procedure specified as follows. Fresh leaf tissue (0.2 g) was homogenized thoroughly in 5 mL of 5% (w/v) trichloroacetic acid (TCA) using a mortar and pestle maintained on ice. The homogenate was centrifuged at 12,000 rpm for 15 min, and the resulting supernatant was collected. An aliquot

of 2 mL of the extract was mixed with an equal volume (2 mL) of 0.5% (w/v) thiobarbituric acid (TBA) prepared in 20% (w/v) TCA. The reaction mixture was incubated at 100 °C for 25 min and then immediately cooled. The samples were subsequently centrifuged at 10,000 rpm for 5 min to remove precipitated material. The absorbance of the resulting supernatant was measured spectrophotometrically at 532 and 600 nm against the appropriate reagent blank. MDA content was calculated from the difference between the absorbance at 532 and 600 nm using the molar extinction coefficient of 155 mM⁻¹ cm⁻¹. The MDA concentration was expressed as nmol g⁻¹ fresh weight (FW) and calculated according to the following equation:

$$\text{MDA } (\mu\text{mol/g MF}) = \frac{(A_{532} - A_{600}) \times V_{\text{extract}}}{155 \times \text{MF(g)}} \times 100 \quad (3)$$

where: A_{532} and A_{600} are the absorbance values measured at 532 and 600 nm, respectively; V is the volume of the extract (mL); 155 mM⁻¹ cm⁻¹ is the molar extinction coefficient of the MDA – TBA complex; and FW is the fresh mass of leaf tissue (g).

Proline content was determined according to (Ábrahám et al., 2010), with the experimental procedure specified as follows. Fresh leaf tissue (0.20 g) was homogenized in 750 µL of 3% (w/v) sulfosalicylic acid using a mortar and pestle. The homogenate was thoroughly vortexed and centrifuged at 14,000 rpm for 15 min at 4 °C. The resulting supernatant (SN1) was collected. The remaining pellet was re-extracted with 750 µL of 3% (w/v) sulfosalicylic acid, thoroughly vortexed, and centrifuged again at 14,000 rpm for 15 min at 4 °C. The second supernatant (SN2) was collected and combined with SN1. For the colorimetric assay, 1 mL of the combined extract (SN1 + SN2) was mixed with 1 mL of toluene and 1 mL of glacial acetic acid. The mixture was thoroughly vortexed and incubated for 1 h at 100 °C. The reaction mixture was then immediately cooled on ice. Subsequently, 2 mL of toluene was added, and the mixture was vortexed. The organic phase was recovered, and its absorbance was measured at 520 nm using a spectrophotometer. Proline concentration was determined from a calibration curve prepared using known concentrations of proline standards and expressed as µg g⁻¹ fresh weight (FW).

Superoxide dismutase and catalase

Superoxide dismutase (SOD) activity was determined according to the photochemical

nitroblue tetrazolium (NBT) reduction method described by (Beauchamp and Fridovich, 1971). Fresh leaf tissue (0.10 g) was homogenized in 10 mL of 0.1 M phosphate buffer (pH 7.8). The homogenate was centrifuged at 12,000 rpm for 20 min at 4 °C, and the resulting supernatant was collected and used as the crude enzyme extract.

SOD activity was assayed in a final reaction volume of 3 mL containing 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM nitroblue tetrazolium (NBT), 0.1 mM EDTA, and 2 µM riboflavin. An aliquot of 50–100 µL of the enzyme extract was added to the reaction mixture. The reaction was initiated by illumination under a fluorescent lamp at approximately 4000 lux for 15 min at 25 °C. A control reaction without enzyme extract was used to determine the maximum photoreduction of NBT, while a non-illuminated reaction mixture was used as the blank. After illumination, the absorbance was measured at 560 nm against the dark blank. SOD activity was calculated based on the inhibition of NBT photoreduction according to the following equation:

$$\text{SOD activity (U)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times \frac{100}{50} \quad (4)$$

where: A_{control} is the absorbance of the illuminated reaction mixture without enzyme extract and A_{sample} is the absorbance of the reaction mixture containing the enzyme extract.

One unit (U) of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT photoreduction under the assay conditions. Total protein concentration was determined using the Bradford method, and SOD activity was normalized to the protein content and expressed as U SOD mg⁻¹ protein.

Catalase (CAT) activity

Fresh leaf tissue (0.10 g) was homogenized in 2 mL of Tris-HCl buffer (pH 7.0) under cold conditions. The homogenate was centrifuged at 4 °C for 20 min, and the resulting supernatant was collected as the crude enzyme extract and kept on ice until analysis.

Catalase activity was determined according to (McKersie et al., 1993), based on the decomposition of hydrogen peroxide (H₂O₂). The reaction mixture (2 mL) contained 50 mM phosphate buffer (pH 7.0), H₂O₂ at a final concentration of 10 mM, and 60 µL of crude enzyme extract. The reaction was initiated by adding the enzyme extract, and the decrease in absorbance at 240 nm was

monitored for 1–3 min. The initial linear decrease in absorbance was used to calculate the reaction rate ($\Delta A_{240} \text{ min}^{-1}$).

CAT activity was calculated using the extinction coefficient of H_2O_2 ($\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) and expressed as $\mu\text{mol H}_2\text{O}_2 \text{ decomposed min}^{-1} \text{ mg}^{-1}$ protein:

$$\text{CAT activity (U)} = \frac{(\Delta \text{Abs}_{240} / \text{min})}{\epsilon (\text{H}_2\text{O}_2 = 39.4 \text{ mM}^{-1} \text{ cm}^{-1})} \times \frac{2}{m \text{ protein}} \quad (5)$$

Multivariate statistical analysis

The disease index and induced protection assay was performed using five independent biological replicates, while biochemical analyses were performed using three independent biological replicates. Results are expressed as mean $\pm$ standard error (SE). Statistical analyses were carried out using SPSS 27.0, with one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test applied for multiple comparisons; differences were considered statistically significant at $p < 0.05$.

Multivariate analysis was further performed using MetaboAnalyst 6.0 (<https://www.metaboanalyst.ca>) (Khallef et al., 2026). Principal component analysis (PCA) was used to evaluate the overall variation among treatments and identify the variables contributing most to their discrimination, while hierarchical cluster analysis (HCA), based on Euclidean distance and Ward's linkage method, illustrated similarities among treatments according to their physiological and biochemical profiles. Pearson correlation analysis was additionally conducted to assess relationships among disease severity, oxidative stress markers, antioxidant enzyme activities, photosynthetic pigments, proline content, and protection rate, with the resulting coefficients displayed as a hierarchical heatmap, where red and blue denoted positive and negative correlations, respectively.

RESULTS

Mycological isolation and molecular identification of *Fusarium oxysporum*

During the mycological investigation, a total of 53 fungal isolates were obtained from tomato roots exhibiting symptoms of Fusarium wilt. The isolates were classified into four different genera, namely *Fusarium*, *Verticillium*, *Rhizoctonia*, and *Phytophthora*. The results indicated a marked predominance of the genus *Fusarium*, which accounted for 72.16% of the total isolates associated with wilt symptoms.

The molecular identification of isolate FO2 was performed by BLASTN analysis of the ITS region. The 351-bp query sequence (GenBank accession PZ939396) was compared against the NCBI Core Nucleotide database. The three top-ranking matches corresponded to *Fusarium oxysporum* and showed 100% query coverage and 100% sequence identity, with an E-value of 0.0. The highest-scoring matches were *F. oxysporum* isolate F6 (OL831176.1), *F. oxysporum* isolate 19 (MN960002.1), and *F. oxysporum* f. sp. *lycopersici* isolate FUS_SR1 (OM618556.2). These BLAST results provide strong molecular support for the identification of isolate FO2 as *Fusarium oxysporum* Table 1.

The sequence of strain FO2 was subsequently deposited in the NCBI GenBank database (<https://www.ncbi.nlm.nih.gov/nucleotide>) under the accession number PZ939396.

Percent disease index (PDI) and induced protection efficacy

To assess the induced resistance in *Fusarium oxysporum*-challenged tomato plants, the efficacy of pretreatment with SA and S applications, either individually or in combination, in reducing wilt disease incidence was evaluated by one-way

Table 1. BLASTN results for the molecular identification of isolate FO2 based on the ITS1–5.8S–ITS2 region

Isolate	GenBank accession (query)	Gene/region	Closest BLAST reference	Reference accession	Query length	Query coverage	Identity	E-value
FO2	PZ939396	ITS1–5.8S–ITS2 (ITS)	<i>Fusarium oxysporum</i> isolate F6	OL831176.1	351 bp	100%	100%	0.0
FO2	PZ939396	ITS1–5.8S–ITS2 (ITS)	<i>Fusarium oxysporum</i> isolate 19	MN960002.1	351 bp	100%	100%	0.0
FO2	PZ939396	ITS1–5.8S–ITS2 (ITS)	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> isolate FUS_SR1	OM618556.2	351 bp	100%	100%	0.0

ANOVA ($n = 5$ biological replicates per treatment; DI %: $F(8, 36) = 29.11$, $p < 0.001$; protection %: $F(8, 36) = 31.84$, $p < 0.001$), followed by Tukey's HSD post-hoc test for pairwise multiple comparisons (Table 2).

The infected control (Infected) showed the highest disease index ($92.00 \pm 4.90\%$), while the healthy control remained symptom free ($0.00 \pm 0.00\%$) and showed complete protection ($100.00 \pm 0.00\%$). The combined treatment (SA+S) was the most effective among the infected treatments, with the lowest disease index ($12.00 \pm 4.90\%$) and the highest protection ($86.96 \pm 5.32\%$), followed by SA3, the highest salicylic acid concentration ($20.00 \pm 6.33\%$ DI; $78.26 \pm 6.87\%$ protection).

Protection increased with concentration for both salicylic acid and sucrose applied alone (SA1 < SA2 < SA3: $43.48\% < 60.87\% < 78.26\%$; S1 < S2 < S3: $17.39\% < 34.78\% < 52.17\%$), confirming a dose-dependent response, with the combined treatment outperforming either compound alone at any concentration. S1, the lowest sucrose concentration, was the least effective treatment, approaching the infected control's disease index ($76.00 \pm 4.00\%$ DI; $17.39 \pm 4.35\%$ protection), with no significant difference from infected ($p = .476$).

Tukey's HSD post-hoc test further clarified the relationships among treatments, revealing a clear gradient from the most protective treatments (SA+S and Control, homogeneous subset "a") to the least protective ones (infected and S1, homogeneous subset "f"). SA+S was statistically indistinguishable from both Control ($p = .803$)

and SA3 ($p = .976$), reflecting near-complete protection, whereas S1 remained statistically similar to the infected control ($p = .476$), confirming its limited protective effect (Table 2) (Figure 1).

Pathogen re-isolation following inoculation

Fungal colonies recovered from the inoculated plants showed morphological features under microscopic examination consistent with those of the originally applied isolates, supporting their role in the observed disease symptoms (Figure 1 and Figure 2).

Biochemical parameters

Photosynthetic pigments

Total chlorophyll content was compared among the nine treatments using one-way ANOVA ($n = 3$ per treatment; $F(8, 18) = 222.388$, $p < 0.001$), followed by Tukey's HSD post-hoc test. *Fusarium oxysporum* infection significantly reduced total chlorophyll content, from $61.34 \pm 0.68 \text{ mg g}^{-1}$ in healthy control plants to $31.59 \pm 0.80 \text{ mg g}^{-1}$ in infected plants, a decrease of approximately 48.5% (Tukey HSD, $p < 0.05$) (Figure 3A). Both sucrose and salicylic acid (SA) treatments significantly alleviated this reduction in a concentration-dependent manner; chlorophyll content increased from $38.50 \pm 0.41 \text{ mg g}^{-1}$ to $44.62 \pm 0.50 \text{ mg g}^{-1}$ and $48.46 \pm 0.58 \text{ mg g}^{-1}$ across the three sucrose concentrations (S1–S3), and from $46.70 \pm 0.68 \text{ mg g}^{-1}$ to $50.25 \pm 0.68 \text{ mg g}^{-1}$ and $53.63 \pm 0.70 \text{ mg g}^{-1}$ across the

Table 2. Effect of the different treatments applied on disease index and induced protection against *Fusarium* wilt in tomato

Treatment	n	DI % (Mean $\pm$ SE)	Protection % (Mean $\pm$ SE)
Infected	5	92.00 ± 4.90^a	0.00 ± 0.00^f
Control	5	0.00 ± 0.00^f	100.00 ± 0.00^a
SA+S	5	12.00 ± 4.90^f	$86.96 \pm 5.32^{a,b}$
SA3	5	$20.00 \pm 6.33^{f,e,d}$	$78.26 \pm 6.87^{b,c}$
SA2	5	$36.00 \pm 7.48^{e,d,c}$	$60.87 \pm 8.13^{c,d}$
S3	5	$44.00 \pm 7.48^{d,c,b}$	$52.17 \pm 8.13^{d,c,b}$
SA1	5	$52.00 \pm 4.90^{c,b}$	43.48 ± 5.32^e
S2	5	$60.00 \pm 6.33^{c,b}$	34.78 ± 6.87^e
S1	5	$76.00 \pm 4.00^{b,a}$	$17.39 \pm 4.35^{f,e}$

Note: Values are expressed as mean $\pm$ standard error (SE, $n = 5$ biological replicates per treatment). Data were analyzed by one-way ANOVA (DI %: $F(8, 36) = 29.11$, $p < 0.001$; protection %: $F(8, 36) = 31.84$, $p < 0.001$), followed by Tukey's HSD post-hoc test; values with different letters within the same column are significantly different ($p < 0.05$).

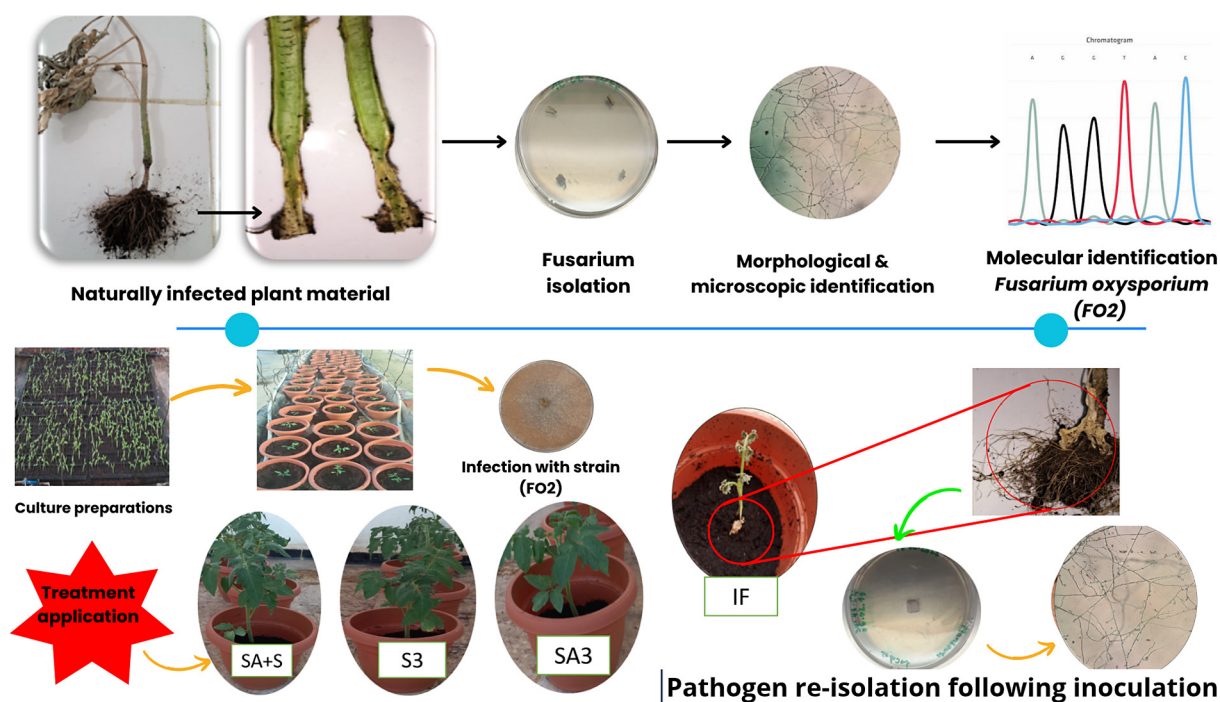


Figure 1. Experimental design: isolation and identification of *Fusarium oxysporum* (FO2), seedling treatment, inoculation, evaluation of Fusarium wilt symptoms, and pathogen re-isolation following inoculation in tomato

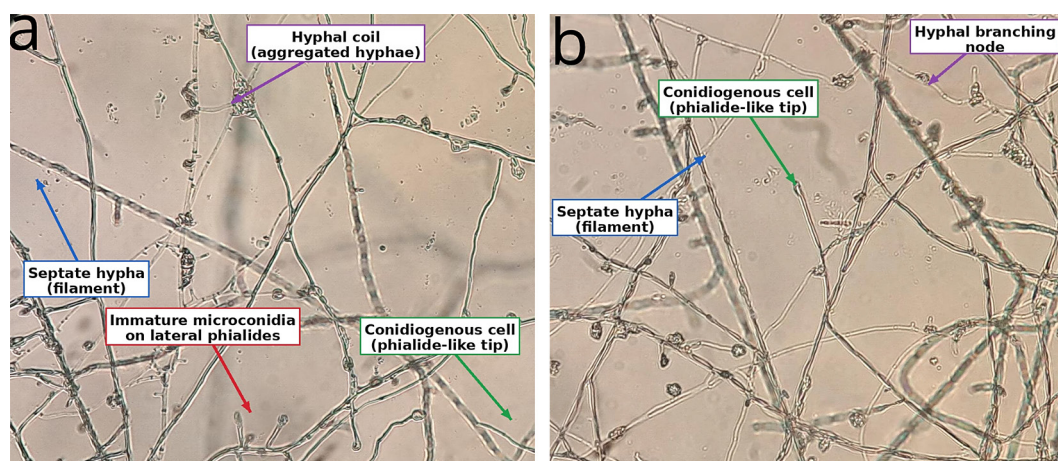


Figure 2. Microscopic comparison of *Fusarium oxysporum* isolate FO2. (a) Original isolate used in this study. (b) Isolate re-obtained from symptomatic tomato tissues after artificial inoculation, showing morphological features consistent with the original isolate (a)

corresponding SA concentrations (SA1–SA3). At each matching concentration level, SA was significantly more effective than sucrose in restoring chlorophyll content ($p < 0.05$). The combined treatment (SA + sucrose) produced the highest chlorophyll content among all treatments ($57.54 \pm 0.42 \text{ mg g}^{-1}$), significantly higher than every individual treatment ($p < 0.05$) and corresponding to nearly 94% of the control level, indicating superior protection of the photosynthetic apparatus against pathogen-induced damage.

Carotenoid content followed a similar pattern and was likewise analyzed by one-way ANOVA ($n = 3$ per treatment; $F(8, 18) = 427.212$, $p < 0.001$), followed by Tukey's HSD post-hoc test (Figure 3B). Infection significantly decreased carotenoid levels from $2.83 \pm 0.03 \text{ mg g}^{-1}$ in control plants to only $0.78 \pm 0.02 \text{ mg g}^{-1}$ in infected plants, a reduction of more than 72% ($p < 0.05$). Both treatments significantly increased carotenoid accumulation in a concentration-dependent manner, from $1.16 \pm 0.06 \text{ mg g}^{-1}$ to $1.32 \pm 0.01 \text{ mg g}^{-1}$ and 1.67

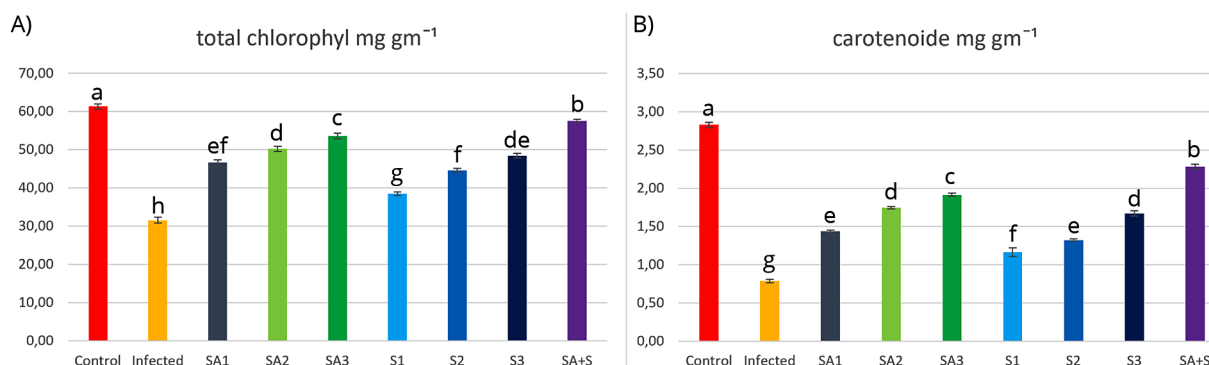


Figure 3. Effect of salicylic acid (SA) and sucrose (S) treatments, applied individually at increasing doses (SA1–SA3, S1–S3) or in combination (SA+S), on photosynthetic pigment content in tomato plants challenged with *Fusarium oxysporum*. (A) Total chlorophyll content (mg·g⁻¹ FW). (B) Carotenoid content (mg·g⁻¹ FW). Control: healthy, non-inoculated plants; Infected: *Fusarium oxysporum*-inoculated, untreated plants. Data are presented as mean ± SE (n = 3 biological replicates per treatment). Data were analyzed by one-way ANOVA followed by Tukey's HSD post-hoc test (total chlorophyll: F(8, 18) = 222.388, p < 0.001; carotenoid content: F(8, 18) = 427.212, p < 0.001); different letters above bars indicate significant differences among treatments (p < 0.05)

± 0.03 mg g⁻¹ across the sucrose concentrations, and from 1.44 ± 0.01 mg g⁻¹ to 1.74 ± 0.01 mg g⁻¹ and 1.92 ± 0.02 mg g⁻¹ across the corresponding SA concentrations. As with chlorophyll, SA significantly outperformed sucrose at each matching concentration (p < 0.05). The combined treatment (SA + sucrose) yielded the highest carotenoid content among all treatments (2.28 ± 0.03 mg g⁻¹), significantly exceeding every individual treatment (p < 0.05) and reaching approximately 81% of the control level, reflecting enhanced protection of the photosynthetic machinery against oxidative stress.

Oxidative stress markers and proline accumulation

H₂O₂ content was compared among the nine treatments using one-way ANOVA (n = 3 per treatment; F(8, 18) = 163.091, p < 0.001), followed by Tukey's HSD post-hoc test. *Fusarium oxysporum* infection significantly increased H₂O₂ content, from 17.43 ± 0.69 μmol g⁻¹ in healthy control plants to 47.27 ± 0.51 μmol g⁻¹ in infected plants (p < 0.05) (Figure 4A). Both sucrose and SA significantly reduced H₂O₂ accumulation in a concentration-dependent manner, with levels declining from 39.72 ± 1.12 μmol g⁻¹ to 32.72 ± 0.43 μmol g⁻¹ across the sucrose concentrations, and from 35.11 ± 0.75 μmol g⁻¹ to 25.71 ± 0.69 μmol g⁻¹ across the corresponding SA concentrations. SA was significantly more effective than sucrose at each matching concentration level (S1 vs SA1, S2 vs SA2, S3 vs SA3; p < 0.05). The combined

treatment (SA + sucrose) provided the strongest protection (21.86 ± 1.06 μmol g⁻¹), significantly lower than every individual treatment (p < 0.05) and corresponding to a 53.8% reduction relative to infected plants, restoring H₂O₂ levels close to those of healthy plants and indicating marked alleviation of pathogen-induced oxidative stress.

Malondialdehyde (MDA) content, an indicator of membrane lipid peroxidation, was analyzed by one-way ANOVA (n = 3 per treatment; F(8, 18) = 146.336, p < 0.001), followed by Tukey's HSD post-hoc test. MDA content increased markedly following *Fusarium oxysporum* infection, from 0.76 ± 0.03 μmol g⁻¹ in control plants to 4.50 ± 0.25 μmol g⁻¹ in infected plants (p < 0.05) (Figure 4B). Both sucrose and salicylic acid significantly reduced MDA accumulation in a concentration-dependent manner, with levels declining from 2.93 ± 0.03 μmol g⁻¹ to 1.99 ± 0.05 μmol g⁻¹ across the sucrose concentrations, and from 2.51 ± 0.05 μmol g⁻¹ to 1.54 ± 0.03 μmol g⁻¹ across the corresponding SA concentrations. SA reduced MDA more than sucrose at the intermediate concentration (S2 vs SA2, p < 0.001), whereas the low and high concentrations did not differ significantly between the two elicitors (S1 vs SA1, p = 0.095; S3 vs SA3, p = 0.062). The combined treatment (SA + sucrose) was the most effective (1.00 ± 0.05 μmol g⁻¹), significantly lower than every individual treatment (p < 0.05) and corresponding to a 77.8% decrease relative to infected plants, indicating a substantial reduction in pathogen-induced membrane oxidative damage. Notably,

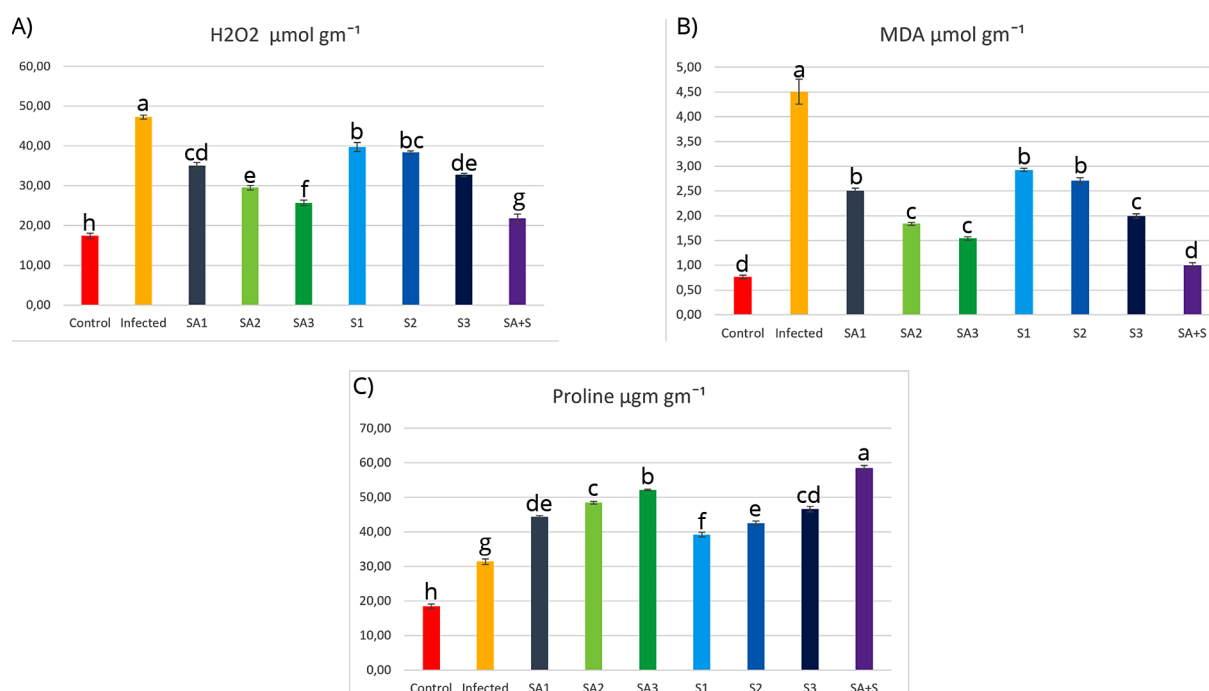


Figure 4. Effect of salicylic acid (SA) and sucrose (S) treatments, applied individually at increasing doses (SA1–SA3, S1–S3) or in combination (SA+S), on oxidative stress markers and osmolyte accumulation in tomato plants challenged with *Fusarium oxysporum*. (A) Hydrogen peroxide (H₂O₂) content (μmol·g⁻¹ FW). (B) Malondialdehyde (MDA) content (μmol·g⁻¹ FW). (C) Proline content (μg·g⁻¹ FW). Control: healthy, non-inoculated plants; Infected: *Fusarium oxysporum*-inoculated, untreated plants. Data are presented as mean ± SE (n = 3 biological replicates per treatment). Data were analyzed by one-way ANOVA followed by Tukey's HSD post-hoc test (H₂O₂: F(8, 18) = 163.091, p < 0.001; MDA: F(8, 18) = 146.336, p < 0.001; proline: F(8, 18) = 391.044, p < 0.001); different letters above bars indicate significant differences among treatments (p < 0.05)

MDA in the combined treatment was not significantly different from the healthy control (p = 0.695), indicating a near-complete restoration of membrane integrity

Proline content was likewise analyzed by one-way ANOVA (n = 3 per treatment; F(8, 18) = 391.044, p < 0.001), followed by Tukey's HSD post-hoc test. In contrast to H₂O₂ and MDA, proline accumulation increased significantly following *Fusarium oxysporum* infection, from 18.52 ± 0.62 μg g⁻¹ in control plants to 31.40 ± 0.80 μg g⁻¹ in infected plants (p < 0.05), and was further enhanced by both sucrose and salicylic acid treatments (Figure 4C). Proline content rose from 39.22 ± 0.68 μg g⁻¹ to 46.61 ± 0.76 μg g⁻¹ across the sucrose concentrations, and from 44.42 ± 0.32 μg g⁻¹ to 52.19 ± 0.17 μg g⁻¹ across the corresponding SA concentrations, with SA producing significantly higher proline accumulation than sucrose at each matching concentration level (p < 0.001). The combined treatment (SA + sucrose) produced the highest proline level (58.55 ± 0.72 μg g⁻¹), significantly exceeding every individual treatment (p < 0.05) and corresponding

to an 86.4% increase relative to infected plants, indicating enhanced osmotic adjustment and improved tolerance to pathogen-induced stress.

Antioxidant enzyme activities

Superoxide dismutase (SOD) activity was compared among the nine treatments using one-way ANOVA (n = 3 per treatment; F(8, 18) = 545.485, p < 0.001), followed by Tukey's HSD post-hoc test. SOD activity increased significantly following *Fusarium oxysporum* infection, from 4.84 ± 0.10 Units mg⁻¹ protein in healthy control plants to 7.75 ± 0.18 Units mg⁻¹ protein in infected plants (p < 0.05) (Figure 5A), and was further enhanced by both sucrose and salicylic acid (SA) treatments in a concentration-dependent manner. SOD activity rose from 8.70 ± 0.07 to 12.36 ± 0.28 Units mg⁻¹ protein across the sucrose concentrations, and from 10.65 ± 0.17 to 14.38 ± 0.12 Units mg⁻¹ protein across the corresponding SA concentrations, with SA inducing significantly greater activity than sucrose at each matching concentration level (S1 vs SA1, S2 vs SA2, S3

vs SA3; $p < 0.05$). The combined treatment (SA + sucrose) produced the highest SOD activity (16.33 ± 0.12 Units mg^{-1} protein), representing a 3.37-fold increase over healthy plants and significantly exceeding every individual treatment, including SA3 ($p < 0.05$). These results indicate that the combined treatment most effectively reinforced the enzymatic antioxidant defense against reactive oxygen species.

Catalase (CAT) activity was analyzed by one-way ANOVA ($n = 3$ per treatment; $F(8, 18) = 704.90$, $p < 0.001$), followed by Tukey's HSD post-hoc test. CAT activity increased significantly following *Fusarium oxysporum* infection, from 18.69 ± 0.89 $\mu\text{mol H}_2\text{O}_2$ oxidized mg^{-1} protein min^{-1} in control plants to 39.15 ± 0.95 $\mu\text{mol mg}^{-1} \text{min}^{-1}$ in infected plants ($p < 0.05$) (Figure 5B), and was further stimulated by both elicitors in a concentration-dependent manner. CAT activity rose from 54.41 ± 0.80 to 69.58 ± 0.44 $\mu\text{mol mg}^{-1} \text{min}^{-1}$ across the sucrose concentrations, and from 62.31 ± 0.82 to 78.57 ± 0.81 $\mu\text{mol mg}^{-1} \text{min}^{-1}$ across the corresponding SA concentrations, with SA producing a significantly stronger response than sucrose at each matching concentration level ($p < 0.05$). The combined treatment (SA + sucrose) resulted in the highest CAT activity (83.36 ± 0.84 $\mu\text{mol mg}^{-1} \text{min}^{-1}$), corresponding to a 4.46-fold increase relative to healthy plants and significantly exceeding every individual treatment, including SA3 ($p < 0.01$), indicating highly efficient detoxification of hydrogen peroxide.

Chemometric analysis of physiological and biochemical data

PCA was performed to explore the multivariate relationships among treatments and physiological variables associated with tomato defense against *Fusarium oxysporum* (Figure 6A). The first two principal components explained 91.3% of the total variance, with PC1 and PC2 accounting for 68.9% and 22.4%, respectively. PC1 clearly separated infected plants (strongly positive values) from control plants and salicylic acid-treated plants, particularly SA3 and the combined treatment (salicylic acid + sucrose), which showed the most negative values; the infected group was closely aligned with the disease index vector (strongly oriented along PC1), as well as the H_2O_2 and MDA vectors, consistent with a pronounced oxidative stress profile. Sucrose-treated plants, as well as SA1 and SA2, occupied intermediate positions along PC1.

PC2 further differentiated, within the «protected» side of PC1 (negative values), two distinct profiles: control plants, strongly positive on PC2, were associated with the protection, total chlorophyll, and carotenoid vectors, whereas SA3 and, most notably, the combined treatment, strongly negative on PC2, were instead associated with the SOD, CAT, and proline vectors. The combined treatment stood out as the most extreme point in the direction of these three vectors, suggesting a particularly strong induction of the antioxidant response, while still maintaining a disease index as low as that of the untreated controls. The

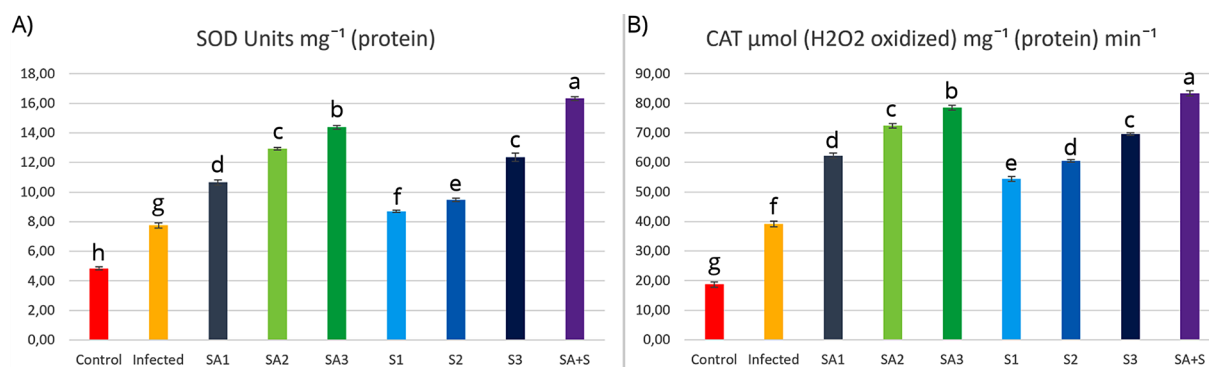


Figure 5. Effect of salicylic acid (SA) and sucrose (S) treatments, applied individually at increasing doses (SA1–SA3, S1–S3) or in combination (SA+S), on antioxidant enzyme activities in tomato plants challenged with *Fusarium oxysporum*. (A) Superoxide dismutase (SOD) activity (units· mg^{-1} protein). (B) Catalase (CAT) activity ($\mu\text{mol H}_2\text{O}_2$ oxidized· mg^{-1} protein· min^{-1}). Data are presented as mean $\pm$ SE ($n = 3$ biological replicates per treatment). Data were analyzed by one-way ANOVA followed by Tukey's HSD post-hoc test (SOD: $F(8, 18) = 545.485$, $p < 0.001$; CAT: $F(8, 18) = 704.90$, $p < 0.001$); different letters above bars indicate significant differences among treatments ($p < 0.05$)

loading vectors additionally confirmed an inverse relationship between oxidative stress markers (disease index, H_2O_2 , MDA) and the variables related to protection, photosynthetic pigments, and antioxidant-related activity.

Hierarchical clustering analysis (HCA) provided a consistent grouping of treatments and variables (Figure 6B). Variables clustered into three well-defined groups: (i) carotenoids, total chlorophyll, and protection rate; (ii) proline, SOD, and CAT; and (iii) MDA, H_2O_2 , and disease index. Infected plants formed a distinct sub-cluster, characterized by the highest MDA, H_2O_2 , and disease index values, together with the lowest pigment content, protection rate, and antioxidant-related activity. Control plants and plants receiving the combined treatment (SA + sucrose), together with some SA3 replicates, grouped into a separate cluster characterized by the lowest MDA, H_2O_2 , and disease index values; within this cluster, control plants stood out for the highest pigment and protection values, whereas the combined treatment and SA3 stood out for the highest SOD, CAT activity and proline accumulation. SA1, SA2, and sucrose treatments (Sucrose

1, 2, 3) displayed a more intermediate and heterogeneous profile, clustering within a larger group alongside the infected plants, with variable but generally lower levels of oxidative stress and disease index than the infected plants themselves.

Pearson correlation analysis supported this three-group structure (Figure 6C). Disease index, H_2O_2 , and MDA were strongly and positively correlated with one another (r close to 1), reflecting a close relationship between disease severity and oxidative damage. Protection rate, total chlorophyll, and carotenoids formed a second cluster, strongly positively correlated within itself and negatively correlated with the first cluster (disease index, H_2O_2 , MDA), suggesting that preservation of photosynthetic pigments is closely linked to reduced oxidative stress and improved resistance. SOD, CAT activity, and proline accumulation formed a third cluster, also strongly positively correlated within itself and negatively correlated with the oxidative stress/disease index cluster. Notably, this third cluster (antioxidant enzymes and proline) was also positively, though more moderately, correlated with the second cluster (protection/pigments), indicating partial

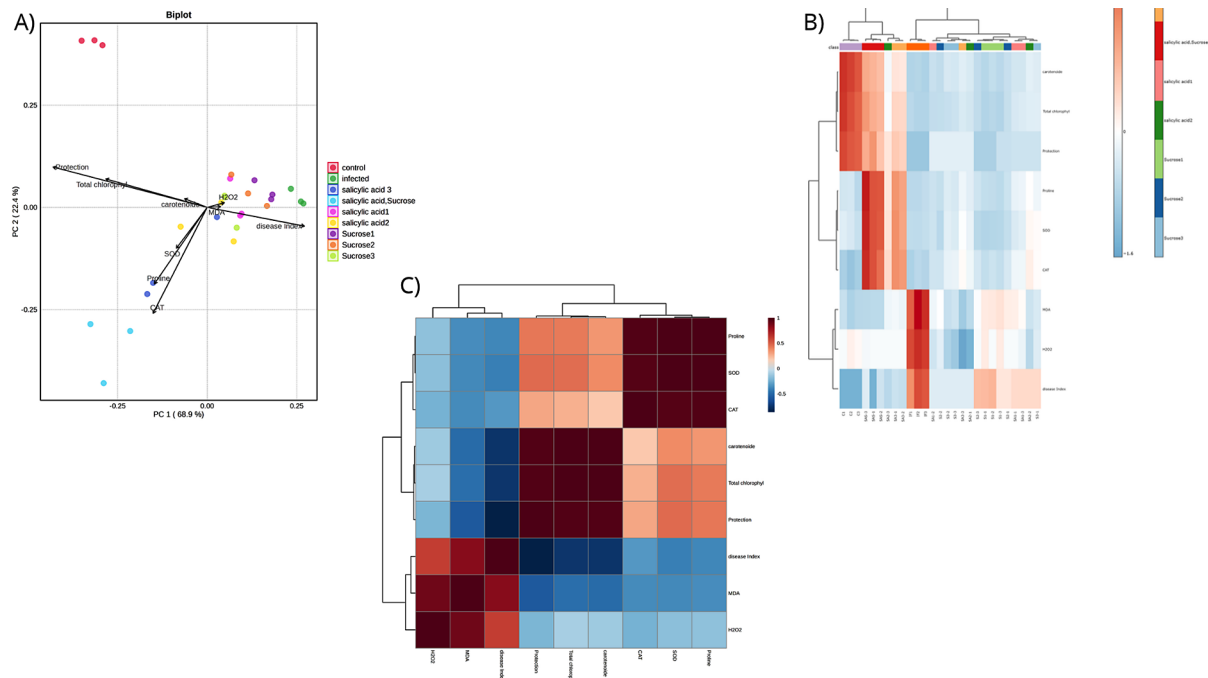


Figure 6. Multivariate analysis of the physiological and biochemical responses of tomato plants treated with salicylic acid (SA), sucrose (Suc), or their combination following inoculation with *Fusarium oxysporum*: (A) PCA showing treatment distribution based on disease index, oxidative stress markers (H_2O_2 , MDA), antioxidant enzymes (SOD, CAT), proline, photosynthetic pigments, and protection rate; (B) hierarchical clustering heatmap of these parameters across treatments after Z-score normalization (red: high; blue: low values); (C) Pearson correlation heatmap among the same variables (red: positive; blue: negative correlation; color intensity reflects correlation strength)

coordination between enzymatic antioxidant activation and pigment/protection preservation, without these two variable sets forming a single unified cluster.

Taken together, these multivariate analyses suggest that the combined application of salicylic acid and sucrose elicited a particularly strong defense response against *Fusarium oxysporum*, combining a reduction in oxidative stress comparable to that of uninfected controls with a marked activation of SOD and CAT activity together with increased proline accumulation, placing this treatment — along with SA3 — among the most effective in limiting disease severity.

DISCUSSION

Tomato vascular wilt caused by *Fusarium oxysporum*, first reported in Florida, USA, at the end of the nineteenth century, remains one of the most destructive diseases affecting tomato production worldwide (Agrios, 2008; Martyn, 2014). In Algeria, *Fusarium oxysporum* is a major soil-borne pathogen responsible for significant yield and fruit quality losses in both greenhouse and open-field cultivation (Azil et al., 2021; Debbi et al., 2018). Although synthetic fungicides are widely used, their effectiveness is compromised by pathogen persistence, reduced sensitivity, and environmental concerns, highlighting the need for sustainable disease management strategies (Li et al., 2022; Urban et al., 2022).

Salicylic acid (SA) is a key phytohormone regulating systemic acquired resistance (SAR) and represents an effective, environmentally friendly strategy for controlling *Fusarium oxysporum*. Exogenous SA has been reported in the literature to enhance tomato resistance by activating antioxidant enzymes and defense-related genes (Li et al., 2022; Zhang et al., 2025). Likewise, carbohydrates such as sucrose, glucose, and trehalose function not only as energy sources but also as signaling molecules that trigger plant defense responses and improve tolerance to biotic and abiotic stresses (Chaliha et al., 2018; Trouvelot et al., 2014).

In the present study, *Fusarium oxysporum* infection caused a pronounced decline in chlorophyll and carotenoid contents, suggesting impairment of the photosynthetic pigment metabolism. Similar reductions have been reported in *Fusarium oxysporum*-infected tomato plants, where

chlorosis and chloroplast degradation are associated with pathogen-induced oxidative stress (Ansari et al., 2025). Excessive production of reactive oxygen species (ROS) during vascular colonization has been reported in the literature to disrupt thylakoid membranes, accelerate chlorophyll degradation, and suppress pigment biosynthesis (Prasad et al., 2023). In the present study, this is consistent with the marked increases in H₂O₂ and malondialdehyde (MDA) observed in infected untreated plants, confirming oxidative stress and membrane lipid peroxidation. Similar elevations in H₂O₂ and MDA have been reported in tomato plants infected with *Fusarium oxysporum*, supporting these compounds as reliable indicators of disease progression (Abdelaziz et al., 2022).

Carotenoids declined more sharply than chlorophyll, which may reflect a greater susceptibility to oxidative degradation, consistent with previous reports linking carotenoid loss to *Fusarium oxysporum*-induced oxidative stress. Comparable observations were reported by (AL-surhane, 2022), who demonstrated that SA and *Trichoderma harzianum* effectively restored photosynthetic pigments in infected tomato plants. Although SOD and CAT activities increased in infected untreated plants, this antioxidant response was insufficient to prevent the elevated H₂O₂ and MDA levels and the associated pigment degradation observed. Similar increases in antioxidant enzyme activities have been described in *Fusarium oxysporum*-infected tomato, suggesting that the endogenous defense system is activated but remains inadequate to fully limit oxidative damage in the absence of protective treatments (Ansari et al., 2025).

Exogenous SA markedly alleviated the detrimental effects of *Fusarium oxysporum* on tomato plants, as evidenced in this study by the dose-dependent preservation of chlorophyll and carotenoid contents and the reduction of the measured oxidative stress markers. These findings are consistent with previous reports indicating that SA helps preserve pigment content and enhances plant resistance to *Fusarium* wilt. Foliar application of SA has been shown to maintain chlorophyll content while significantly reducing disease incidence in infected tomato plants (Chakraborty, 2021). Likewise, increasing SA concentrations have been reported to provide progressively stronger protection of photosynthetic pigments, with the highest concentration producing the greatest improvement in chlorophyll and carotenoid levels (Heikal et al., 2025).

The progressive decline in H_2O_2 and MDA observed from SA1 to SA3 further demonstrates that SA effectively limits *Fusarium oxysporum*-induced oxidative stress. The measured increases in SOD and CAT activity indicate a reinforced enzymatic antioxidant capacity in SA-treated plants, consistent with reports of enhanced antioxidant enzyme activity following SA treatment in tomato infected with *Fusarium* species (Ansari et al., 2025; Mufti et al., 2023). SOD is understood to convert superoxide radicals into H_2O_2 , while CAT decomposes H_2O_2 into water and oxygen, together contributing to the detoxification of reactive oxygen species. Furthermore, the positive relationship between enhanced antioxidant activity and chlorophyll preservation reported by (Li et al., 2022) is consistent with the physiological responses observed in the present study.

Sucrose treatment also improved plant tolerance to *Fusarium oxysporum*, although its protective effects remained less pronounced than those induced by SA. Besides serving as a carbon source, sucrose has been proposed in the literature to function as a signaling molecule involved in stress adaptation and antioxidant metabolism, although this signaling role was not directly assessed here. Increased chlorophyll accumulation and improved plant performance following sucrose application have been reported under abiotic stress conditions (Sevgi and Leblebici, 2025) a major ecological problem worldwide, adversely affects plant growth and productivity. Osmoprotectants are a possible strategy for plants to cope with and regulate their response to unfavorable environmental conditions, such as salinity. However, the role of sucrose in this process requires more precise elucidation. This study aims to investigate the ameliorative role of sucrose on growth parameters, proline content, antioxidant enzyme activity, and gene expression in sunflower and canola under salt stress. The treatments included a 3% sucrose concentration and two levels of salinity (75 and 150 mM NaCl). Consistent with this, the enhanced SOD and CAT activities observed in sucrose-treated plants were accompanied by lower H_2O_2 and MDA accumulation and improved preservation of photosynthetic pigments. Nevertheless, the lower efficiency of sucrose compared with SA suggests that exogenous SA provided more effective protection against *Fusarium oxysporum* infection than sucrose applied alone.

The combined treatment (salicylic acid and sucrose) produced the strongest protective effect against *Fusarium oxysporum*, resulting in the highest activities of SOD and CAT, the greatest chlorophyll and carotenoid contents, the lowest H_2O_2 and MDA accumulation, and the highest proline levels among all infected treatments. These results indicate that the effect of the combined treatment exceeded that of either compound applied alone. Similar observations were reported by (AL-surhane, 2022), who demonstrated that the combined application of SA and *Trichoderma harzianum* provided greater protection against *Fusarium* wilt than either treatment applied alone.

The present study based strictly on the physiological and biochemical parameters measured here, the combined treatment achieved the greatest reduction in H_2O_2 and MDA together with the highest SOD and CAT activities, indicating more effective mitigation of oxidative stress than either elicitor applied alone.

Proline accumulation further supported the enhanced stress tolerance induced by the combined treatment. While *Fusarium oxysporum* infection alone increased proline, likely as a response to vascular dysfunction and osmotic stress, the combined treatment significantly amplified its accumulation. Similar increases in proline have been reported in *Fusarium oxysporum*-infected tomato plants treated with plant growth-promoting bacteria, where elevated proline was associated with improved stress adaptation (Ansari et al., 2025). Overall, the combined action of SA and sucrose provided the most effective antioxidant, osmotic, and photosynthetic protection against *Fusarium oxysporum* infection, maintaining pigment contents close to those of healthy plants.

CONCLUSIONS

Under greenhouse conditions, the exogenous application of SA and sucrose significantly enhanced tomato resistance against *Fusarium oxysporum*. Both elicitors improved antioxidant enzyme activities (SOD and CAT), reduced H_2O_2 and MDA accumulation, preserved chlorophyll and carotenoid contents, and promoted proline accumulation, thereby mitigating oxidative damage and maintaining plant physiological performance. Salicylic acid consistently outperformed sucrose when applied alone, whereas their combined application provided the greatest

protection, producing the lowest disease index and the strongest activation of the measured defense responses. These findings indicate the potential of the combined SA and sucrose treatment as an eco-friendly strategy for managing Fusarium wilt and support its further evaluation under field conditions to optimize application strategies for sustainable tomato production.

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