



Dijlah Journal of Agricultural Sciences

Journal homepage: <https://djas.uowasit.edu.iq/index.php/djas/en>



Research article

Evaluating the Potential of *Aspergillus niger* and Salicylic Acid to Induce Resistance against Fusarium-Associated Wilt in Watermelon

Muntaha Hashim Mansour and Azher Hameed Al-Taie

Department of Plant Protection, College of Agriculture, Wasit University, Wasit 52001, Iraq

ARTICLE INFO

Keywords:

Watermelon;
Fusarium wilt;
Aspergillus niger;
Salicylic acid;
Induced resistance;
Peroxidase activity.

Article history:

Received: 23 Aug 2026
Accepted: 9 Sep 2026
Online: 30 Sept 2026

ABSTRACT

This study evaluated the potential of salicylic acid (SA) and *Aspergillus niger* culture filtrate to induce resistance against Fusarium-associated wilt in two watermelon cultivars, Sugar Baby and Aseel, under greenhouse conditions. Molecular identification based on ITS sequencing identified *Fusarium proliferatum*, *F. solani*, and *F. oxysporum* among the recovered isolates. Disease incidence reached 100% in plants inoculated with *F. oxysporum* and *F. solani*, whereas *F. proliferatum* caused 66.67% incidence in Sugar Baby and 100% in Aseel. Application of *A. niger* or SA markedly reduced disease incidence, with the lowest value of 23.33% recorded in Sugar Baby treated with *F. oxysporum* + *A. niger* or *F. oxysporum* + SA, and in Aseel treated with *F. proliferatum* + *A. niger*. *A. niger* alone produced the highest growth values in Sugar Baby, including 0.17 mm stem diameter, 17.20 cm root length, 25.00 cm plant height, 16.22 SPAD chlorophyll content, and 2.52 cm² leaf area. In Aseel, the corresponding maximum values were 12.90 cm root length, 21.47 cm plant height, 10.27 SPAD, and 1.04 cm² leaf area. Peroxidase activity was enhanced in combined treatments, reaching 0.82 μmol tetra-guaiacol min⁻¹ g⁻¹ FW in Sugar Baby treated with *F. oxysporum* + SA and 0.79 μmol tetra-guaiacol min⁻¹ g⁻¹ FW in Aseel treated with *F. proliferatum* + *A. niger*. Overall, SA and *A. niger* reduced disease development and improved several growth and defense-related traits, although their effectiveness varied according to the watermelon cultivar and *Fusarium* species.

1. Introduction

Watermelon (*Citrullus lanatus*) is an economically and nutritionally important crop widely cultivated throughout the world. However, its production is constrained by several diseases, among which Fusarium wilt is considered one of the most destructive soil-borne diseases affecting watermelon. The disease is primarily caused by *Fusarium oxysporum* f. sp. *niveum* (FON) and occurs in major watermelon-producing regions worldwide. Severe infections can substantially reduce plant growth and yield, particularly under favorable environmental conditions and continuous or intensive watermelon cultivation (Everts and Himmelstein, 2015; Rahman et al., 2021). The persistence of the pathogen in soil and the emergence of more virulent races further complicate disease management, emphasizing the need for effective and sustainable control strategies. Although *F. oxysporum* is the primary causal agent of classical Fusarium wilt in watermelon, other *Fusarium* species may also be associated with root, crown, and wilt symptoms. Therefore, the term "Fusarium-associated wilt" was used in the present study to describe the disease complex associated with the recovered *Fusarium* species.

✉ *Corresponding author:
aaltaie@uowasit.edu.iq (Azher Hameed Al-Taie)

Chemical fungicides are commonly used to manage fungal wilt diseases and maintain watermelon productivity. In addition, some cultivars possess varying levels of genetic resistance to specific plant pathogens, which may contribute to reducing the intensive use of fungicides (Dauda et al., 2024). Consequently, considerable research attention has been directed toward understanding plant-pathogen interactions and identifying defense mechanisms associated with host resistance (Wang et al., 2015; Zhang and Li, 2019). Activation of the plant's natural defense mechanisms represents an important approach to sustainable plant disease management because it can reduce dependence on chemical pesticides and minimize their adverse environmental effects. Among these approaches is the use of chemical elicitors and biological agents capable of promoting plant growth and activating natural defense responses (du Jardin, 2015).

Salicylic acid is one of the major compounds involved in the regulation of plant defense responses and plays an important role in activating several mechanisms associated with induced resistance (Koo et al., 2020). Certain fungi, including *Aspergillus niger*, or metabolites present in their culture filtrates may also contribute to plant growth promotion and the enhancement of physiological and defense-related responses. Peroxidase is an important enzyme associated with plant responses to biotic stress. It contributes to the regulation of reactive oxygen species, polymerization of phenolic compounds, and lignin deposition in cell walls, thereby strengthening plant tissues and restricting pathogen spread. Therefore, peroxidase activity is commonly used as an indicator of induced resistance (Yang et al., 2022).

Accordingly, this study aimed to evaluate the efficiency of salicylic acid and *Aspergillus niger* culture filtrate in inducing resistance in watermelon plants against Fusarium-associated wilt and improving selected growth and defense-related parameters under greenhouse conditions. The study also evaluated the effects of the different treatments on reducing disease incidence and identified the most effective treatments for potential use in integrated disease management programs.

2. Materials and Methods

2.1. Isolation, Purification, and Identification of Fungi Associated with Vascular Wilt Symptoms

Samples were collected from watermelon fields in Kut City, Wasit Governorate, Iraq. Watermelon plants showing typical symptoms of vascular wilt, including wilting, yellowing, and vascular discoloration in the crown and lower stem regions, were selected for fungal isolation. The collected samples were placed in polyethylene bags and transported to the laboratory for further examination. Portions of the lower stems, crowns, and roots were excised and washed thoroughly under running tap water to remove adhering soil particles and other debris. The plant tissues were then surface-disinfected with sodium hypochlorite solution for 2 min and subsequently rinsed with sterile distilled water.

The disinfected plant tissues were cut into small pieces approximately 0.5 cm in length and transferred onto Petri dishes containing Water Agar (WA) medium. The plates were incubated at 25°C for 3 days. Emerging fungal colonies were examined microscopically and subcultured for further purification and identification.

Fragments from the developing fungal colonies on WA medium were transferred onto Petri dishes containing Potato Dextrose Agar (PDA). The medium was prepared at a concentration of 40 g L⁻¹ using commercially prepared PDA and distilled water. The medium was autoclaved at 121°C for 15 min. After autoclaving, the medium was allowed to cool before tetracycline was added to a final concentration of 200 mg L⁻¹, and the medium was then poured into sterile Petri dishes.

Pure fungal cultures were obtained using the single-spore technique. The plates were incubated at 25°C for 5–6 days. After confirming the purity of the isolates, they were identified morphologically based on their cultural and microscopic characteristics, including colony morphology, pigmentation,

and the morphological characteristics of macroconidia, microconidia, and other diagnostic structures, according to Booth (1971).

Pure isolates were maintained in test tubes containing sterile PDA slants and incubated at 25°C for 7 days. After sufficient fungal growth had developed, the cultures were stored at 4°C until required for subsequent experiments.

2.2. Molecular Identification of *Fusarium* Isolates

2.2.1. Genomic DNA Extraction

Genomic DNA was extracted from fungal mycelia using the Presto™ Mini gDNA Yeast Kit (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's instructions, with minor modifications suitable for filamentous fungi. Fungal mycelium was collected from 5–7-day-old cultures grown on PDA and transferred into sterile microcentrifuge tubes containing ceramic beads to facilitate mechanical disruption of the fungal cell walls.

The fungal mycelium was mechanically homogenized until a uniform suspension was obtained. Subsequently, 600 µL of GT Buffer and 20 µL of Proteinase K were added to each sample. The samples were thoroughly mixed using a vortex mixer and incubated in a water bath at 60°C for 10 min. Following incubation, the samples were vortexed again to ensure adequate disruption and lysis of the fungal cells.

The lysates were centrifuged at 13,000 × g for 3 min to remove fungal cell debris and incompletely disrupted mycelial material. The resulting clear supernatant was carefully transferred into new sterile microcentrifuge tubes without disturbing the pellet.

A volume of 450 µL of absolute ethanol was then added to each sample and mixed thoroughly by vortexing to facilitate DNA binding to the silica membrane. The mixture was transferred to the spin column supplied with the kit and centrifuged at 12,000 × g for 1 min. The flow-through was discarded, and the procedure was repeated until the entire sample had passed through the column.

The column was washed using the washing buffers supplied with the extraction kit according to the manufacturer's instructions. Following the final washing step, the column was centrifuged to remove residual ethanol and wash buffer. Genomic DNA was subsequently eluted by adding 100 µL of Elution Buffer to the center of the silica membrane, allowing it to stand for 2 min, and then centrifuging to recover the purified DNA.

The quality and integrity of the extracted genomic DNA were evaluated by electrophoresis on a 1% agarose gel. The purified DNA was stored at –20°C until use in PCR amplification.

2.2.2. Polymerase Chain Reaction (PCR)

The Internal Transcribed Spacer (ITS) region of the ribosomal DNA was amplified by polymerase chain reaction (PCR) using the universal fungal primers ITS1 and ITS4 (White et al, 1990). The primers were obtained in lyophilized form from Metabion International and reconstituted according to the manufacturer's instructions (Table 1).

Table 1. Primers used for amplification of the ITS region of *Fusarium* isolates

Primer	Primer sequence (5'–3')	Target region	Expected amplicon size
ITS1	TCCGTAGGTGAACCTGCGG	ITS1–5.8S–ITS2	Approximately 500–650 bp
ITS4	TCCTCCGCTTATTGATATGC	ITS1–5.8S–ITS2	Approximately 500–650 bp

PCR amplification was performed in a final reaction volume of 20 µL. Each reaction contained 1 µL of ITS1 forward primer (10 pmol µL⁻¹), 1 µL of ITS4 reverse primer (10 pmol µL⁻¹), 5 µL of genomic DNA template, and 13 µL of nuclease-free water. The components were added directly to tubes containing lyophilized AccuPower® PCR PreMix. The final concentration of each primer in the reaction mixture was 0.5 µM.

The reaction mixtures were gently mixed by vortexing for approximately 5 s and briefly centrifuged for 10 s to collect all components at the bottom of the tubes.

The ITS region of the selected *Fusarium* isolates was amplified using a thermal cycler. PCR amplification was performed with an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. A final extension was carried out at 72°C for 5 min, followed by holding at 4°C.

Following amplification, the PCR products were separated by electrophoresis on a 1% agarose gel prepared in 1× TAE buffer. A suitable DNA molecular-weight marker was electrophoresed alongside the PCR products to estimate the size of the amplified fragments. The DNA bands were visualized using a gel documentation system, and products showing a single band of the expected size were selected for subsequent sequencing and molecular identification.

2.3. Preparation of *Fusarium* Inoculum and *Aspergillus niger* Culture Filtrate

Selected *Fusarium* isolates were reactivated on Petri dishes containing PDA medium and incubated at 25°C for 8 days in darkness. Microconidia were gently harvested from the surfaces of the fungal colonies using sterile distilled water.

The resulting spore suspension was filtered through four layers of cheesecloth to remove residual mycelial fragments. The concentration of the conidial suspension was adjusted to 1×10^6 conidia mL⁻¹ using a hemocytometer. The prepared suspension was subsequently used to inoculate the growing medium in the greenhouse experiment, as described by Kasote et al. (2020).

A. niger was cultured on potato dextrose agar (PDA) plates and incubated at 25°C for 7 days. A 5-mm-diameter mycelial disc taken from the actively growing margin of the colony was transferred into a 250-mL Erlenmeyer flask containing 100 mL of sterile potato dextrose broth (PDB). The cultures were incubated at 25°C for 7 days on a rotary shaker at 150 rpm. After incubation, the culture was filtered through sterile cheesecloth followed by Whatman No. 1 filter paper to remove fungal mycelia and spores. The filtrate was then centrifuged at $7,000 \times g$ for 15 min, and the resulting supernatant was collected as the cell-free culture filtrate. A 50% (v/v) concentration was prepared by mixing equal volumes of the culture filtrate and sterile distilled water. The prepared filtrate was used immediately in the greenhouse experiment.

2.4. Greenhouse Experiment for Evaluation of Induced Resistance Against *Fusarium*-associated wilt

The greenhouse experiment was conducted at the College of Agriculture, University of Wasit, Wasit Governorate, Iraq, to evaluate the potential of *A. niger* and salicylic acid (SA) to induce resistance against *Fusarium*-associated wilt in watermelon.

Seeds of two watermelon (*Citrullus lanatus*) cultivars, Sugar Baby and Aseel, were obtained from local markets. Sugar Baby was considered susceptible to *Fusarium*-associated wilt, whereas Aseel was considered moderately susceptible.

The experiment was carried out in plastic growing bags (30 × 30 × 30 cm) containing sterilized peat moss prepared according to Tawajin (1979). Each experimental unit consisted of one growing bag containing three watermelon plants, and each treatment was replicated three times. The growing medium was inoculated by applying 5 mL of the conidial suspension to each designated planting site, as described in Section 2.3.

Three days after inoculation with the *Fusarium* isolates, the designated treatments were inoculated with a 50% culture filtrate of *A. niger*. The treated growing medium was then maintained for an additional 3 days before transplanting the watermelon seedlings.

Uniform seedlings of both watermelon cultivars at the three-true-leaf stage were used for transplantation. For treatments involving salicylic acid, the roots of watermelon seedlings at the three-true-leaf stage were immersed in a 100 µM salicylic acid solution for 20 min immediately

before transplantation. Twenty-four hours after transplantation, an additional 5 mL of 100 μ M salicylic acid solution was applied to the rhizosphere of each designated plant. This concentration and treatment method were selected based on the effectiveness of exogenous salicylic acid in enhancing watermelon resistance to *Fusarium* wilt (Zhu et al., 2022).

The experiment consisted of 24 treatments representing the two watermelon cultivars and the designated combinations of *F. oxysporum*, *F. proliferatum*, *F. solani* isolates, *A. niger* culture filtrate (AS), and salicylic acid (SA). The experiment was arranged in a randomized complete block design (RCBD) with three replicates per treatment. All plants were maintained under the same greenhouse conditions and received uniform irrigation and cultural practices throughout the experimental period.

The effects of the treatments were evaluated 40 days after transplantation by measuring the following growth parameters: leaf area (cm^2) according to Lotfi (1986), chlorophyll content using a SPAD meter, plant height (cm), root length (cm), stem diameter (mm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), and root dry weight (g).

2.5. Assessment of Shoot and Root Growth Parameters

Forty days after transplantation, the effects of the different treatments on watermelon growth were evaluated by measuring a series of shoot and root growth parameters. Leaf area was determined and expressed as $\text{cm}^2 \text{ plant}^{-1}$ according to Lotfi (1986). Leaf chlorophyll content was measured using a SPAD chlorophyll meter. Plant height and root length were measured in centimeters, whereas stem diameter was measured in millimeters. For biomass determination, shoots and roots were separated carefully and weighed immediately to determine their fresh weights (g plant^{-1}). The plant materials were then dried in an oven until a constant weight was obtained, after which shoot and root dry weights were recorded and expressed as g plant^{-1} .

Fusarium-associated wilt incidence was assessed 40 days after transplantation based on the appearance of typical wilt symptoms, including leaf yellowing, wilting, and plant collapse. Disease incidence was calculated for each treatment using the following equation:

$$\text{Disease incidence (\%)} = (\text{Number of symptomatic plants} / \text{Total number of plants evaluated}) \times 100$$

The calculated disease incidence values were used to compare the effects of the different treatments on the development of *Fusarium*-associated wilt.

2.6. Determination of Peroxidase Activity

Peroxidase (POD; EC 1.11.1.7) activity was determined in watermelon roots as a biochemical indicator of induced resistance according to Matroud (2015). Root samples were collected separately from each treatment, placed in labeled polyethylene bags, immediately transferred to an ice-cooled container, and transported to the laboratory for enzyme extraction.

For enzyme extraction, 150 mg of fresh root tissue was washed with deionized distilled water, gently blotted dry, and homogenized in 2.5 mL of ice-cold 0.05 M potassium phosphate buffer (pH 6.0). The homogenates were centrifuged at $12,000 \times g$ for 20 min at 4°C , and the resulting clear supernatant was collected and used immediately as the crude enzyme extract. Peroxidase activity was determined spectrophotometrically using guaiacol as the electron donor and hydrogen peroxide (H_2O_2) as the substrate, according to Chance and Maehly (1955).

The reaction mixture consisted of 2.5 mL of 0.05 M potassium phosphate buffer (pH 6.0), 250 μ L of 0.5% guaiacol, 250 μ L of freshly prepared 0.3% H_2O_2 , and 100 μ L of crude enzyme extract.

The reaction was initiated by the addition of the enzyme extract, and the increase in absorbance resulting from the oxidation of guaiacol to tetra-guaiacol was monitored at 470 nm using a spectrophotometer. Absorbance was recorded at 20-s intervals for 1 min, and the linear change in absorbance per minute ($\Delta A_{470} \text{ min}^{-1}$) was used to calculate enzyme activity. A reaction mixture containing all components except the enzyme extract was used as a blank. Three biological replicates were analyzed for each treatment.

Peroxidase activity was calculated using the molar extinction coefficient of tetra-guaiacol at 470 nm ($\epsilon = 26.6 \text{ mM}^{-1} \text{ cm}^{-1}$) according to Chance and Maehly (1955) and expressed as $\mu\text{mol tetra-guaiacol min}^{-1} \text{ g}^{-1}$ fresh weight (FW) using the following equation:

$$\text{POD activity } (\mu\text{mol min}^{-1} \text{ g}^{-1} \text{ FW}) = (\Delta A_{470}/\text{min} \times V_r \times V_e) / (\epsilon \times l \times V_s \times W)$$

where $\Delta A_{470}/\text{min}$ is the change in absorbance at 470 nm per minute; V_r is the total reaction volume (3.10 mL); V_e is the total volume of enzyme extract (2.5 mL); V_s is the volume of enzyme extract used in the assay (0.10 mL); ϵ is the extinction coefficient of tetra-guaiacol ($26.6 \text{ mM}^{-1} \text{ cm}^{-1}$); l is the optical path length of the cuvette (1 cm); and W is the fresh weight of root tissue used for extraction (0.15 g). The resulting values were used to compare peroxidase activity among the different treatments as an indicator of biochemical defense responses induced in watermelon plants.

2.7. Statistical Analysis

Experimental data were analyzed using GenStat statistical software. Treatment means were compared using the Least Significant Difference (LSD) test at the 5% probability level according to Al-Rawi and Khalaf Allah (1980). Microsoft Excel was also used for data organization and graphical presentation.

3. Results and Discussion

3.1 Molecular Identification of *Fusarium* spp. Isolates

PCR amplification of the ITS1–5.8S–ITS2 region using the universal primers ITS1 and ITS4 produced clear DNA amplicons of approximately 652 bp for the selected *Fusarium* isolates. The amplified products were subjected to nucleotide sequencing, and the resulting sequences were compared with reference sequences available in the NCBI GenBank database using the BLASTn algorithm. Sequence comparison, together with cultural and microscopic characteristics, supported the identification of the recovered *Fusarium* isolates. ITS sequence analysis showed the highest similarity to *Fusarium proliferatum*, *F. solani*, and *F. oxysporum*. The sequences identified as *F. proliferatum*, *F. solani* and *F. oxysporum* were deposited in GenBank under accession numbers PZ372941, PZ372942 and PZ372944 respectively. Overall, The ITS sequence analysis and BLASTn comparison, together with the cultural and microscopic characteristics, provided molecular support for the identification of the studied *Fusarium* isolates.

3.2 *Fusarium*-associated Wilt Disease Incidence

Fusarium-associated wilt incidence differed significantly among the tested treatments (Table 2). No disease symptoms were observed in the control, *A. niger*-only, or salicylic acid-only treatments in either cultivar, and disease incidence remained at 0%, indicating that these treatments did not cause wilt symptoms in the absence of *Fusarium* inoculation. Inoculation with the *Fusarium* species resulted in a marked increase in disease incidence. *Fusarium oxysporum* and *F. solani* caused 100% disease incidence in both Sugar Baby and Aseel. Similarly, *F. proliferatum* caused 100% incidence in

Aseel, whereas disease incidence in Sugar Baby reached 66.67%, indicating variation in cultivar response to this pathogen. Application of *A. niger* markedly reduced Fusarium-associated wilt incidence. In Sugar Baby, disease incidence decreased to 23.33, 26.67, and 33.33% when *A. niger* was combined with *F. oxysporum*, *F. proliferatum*, and *F. solani*, respectively. In Aseel, the corresponding disease incidences were 30.00, 23.33, and 46.67%, respectively.

Salicylic acid also substantially reduced disease development. In Sugar Baby, disease incidence was reduced to 23.33% with *F. oxysporum*, 30.00% with *F. proliferatum*, and 36.67% with *F. solani*. In Aseel, the corresponding values were 26.67, 36.67, and 43.33%, respectively. Among the pathogen-resistance inducer combinations, the lowest disease incidence (23.33%) was recorded in Sugar Baby treated with *F. oxysporum* + *A. niger*, Sugar Baby treated with *F. oxysporum* + salicylic acid, and Aseel treated with *F. proliferatum* + *A. niger*. In contrast, the highest residual disease incidence among the resistance-inducing treatments was 46.67% in Aseel inoculated with *F. solani* and treated with *A. niger*.

Overall, both *A. niger* and salicylic acid were effective in suppressing Fusarium-associated wilt compared with pathogen-only treatments. However, their effectiveness varied according to the *Fusarium* species and watermelon cultivar, suggesting an interaction between pathogen species, host genotype, and resistance-inducing treatment. The LSD values were 2.26 for Sugar Baby and 3.64 for Aseel, indicating that the large reductions observed following *A. niger* and salicylic acid treatments were greater than the minimum differences required for statistical significance at the adopted probability level.

Table 2. Fusarium-associated wilt incidence (%) in watermelon seedlings at 40 days after transplantation

Treatment	Sugar Baby Disease incidence (%)	Aseel Disease incidence (%)
Control	0	0
<i>A. niger</i>	0	0
Salicylic acid	0	0
<i>F. oxysporum</i>	100	100
<i>F. proliferatum</i>	66.67	100
<i>F. solani</i>	100	100
<i>F. oxysporum</i> + <i>A. niger</i>	23.33	30
<i>F. proliferatum</i> + <i>A. niger</i>	26.67	23.33
<i>F. solani</i> + <i>A. niger</i>	33.33	46.67
<i>F. oxysporum</i> + Salicylic acid	23.33	26.67
<i>F. proliferatum</i> + Salicylic acid	30	36.67
<i>F. solani</i> + Salicylic acid	36.67	43.33
L.S.D	2.26	3.64

3.3 Effect of Treatments on Shoot and Root Growth Parameters of Watermelon Seedlings

The tested treatments markedly affected the vegetative growth parameters of both watermelon cultivars, Sugar Baby and Aseel. In general, inoculation with *Fusarium* spp. reduced plant growth, whereas treatment with *A. niger* or salicylic acid (SA), particularly when applied alone, improved several growth parameters compared with the untreated control.

In the Sugar Baby cultivar, *A. niger* alone produced the highest values for most growth parameters. Stem diameter, root length, plant height, chlorophyll content, and leaf area reached 0.17 mm, 17.20 cm, 25.00 cm, 16.22 SPAD, and 2.52 cm² plant⁻¹, respectively, compared with 0.15 mm, 14.23 cm, 20.80 cm, 13.08 SPAD, and 1.92 cm² plant⁻¹ in the control treatment. Salicylic acid alone also improved plant growth, particularly plant height, chlorophyll content, and leaf area, which reached 23.60 cm, 14.40 SPAD, and 2.22 cm² plant⁻¹, respectively.

In contrast, inoculation with the three *Fusarium* species markedly reduced most growth parameters in Sugar Baby. Among the pathogen-only treatments, *F. solani* caused the greatest reduction in several traits, with root length, plant height, chlorophyll content, and leaf area decreasing to 10.27 cm, 14.57 cm, 3.33 SPAD, and 0.69 cm² plant⁻¹, respectively. Plants inoculated with *F. proliferatum* or *F. oxysporum* also showed substantial reductions compared with the control.

Application of *A. niger* or SA to *Fusarium*-inoculated plants partially alleviated the adverse effects of the pathogens. For example, chlorophyll content in plants inoculated with *F. proliferatum* increased from 3.57 SPAD to 12.47 SPAD following treatment with *A. niger*. Similarly, the addition of SA to *F. oxysporum*-inoculated plants increased root length from 11.30 to 13.90 cm, plant height from 17.37 to 19.27 cm, chlorophyll content from 4.00 to 8.10 SPAD, and leaf area from 0.75 to 1.66 cm² plant⁻¹. The combination of *F. solani* with SA also improved plant height and leaf area to 19.90 cm and 1.83 cm² plant⁻¹, respectively, compared with 14.57 cm and 0.69 cm² plant⁻¹ in plants inoculated with *F. solani* alone.

A similar overall response was observed in the Aseel cultivar. The highest growth values were generally obtained with *A. niger* alone, which resulted in a root length of 12.90 cm, plant height of 21.47 cm, chlorophyll content of 10.27 SPAD, and leaf area of 1.04 cm² plant⁻¹. Salicylic acid alone also improved chlorophyll content to 8.77 SPAD, compared with 6.40 SPAD in the control.

The three *Fusarium* species caused a pronounced reduction in Aseel growth. Plant height decreased to 8.87, 8.93, and 8.37 cm following inoculation with *F. proliferatum*, *F. oxysporum*, and *F. solani*, respectively, compared with 19.77 cm in the control. Root length similarly declined to 6.60–6.83 cm, while chlorophyll content declined to 1.75–2.42 SPAD.

Treatment with *A. niger* substantially reduced the growth-suppressive effects of the pathogens in Aseel. Root length increased from 6.60 to 10.37 cm in *F. proliferatum*-inoculated plants, from 6.83 to 11.27 cm in *F. oxysporum*-inoculated plants, and from 6.47 to 11.27 cm in *F. solani*-inoculated plants. Because these differences exceeded the LSD value for root length (1.611 cm), they were statistically significant.

The improvement in plant height following *A. niger* treatment was particularly pronounced. Plant height increased from 8.87 to 16.57 cm with *F. proliferatum*, from 8.93 to 17.40 cm with *F. oxysporum*, and from 8.37 to 18.30 cm with *F. solani*. These differences exceeded the LSD value of 3.595 cm, indicating a significant recovery of plant growth.

Among the combined treatments in Aseel, *F. solani* + *A. niger* produced a particularly strong response, increasing chlorophyll content from 1.75 to 6.12 SPAD and leaf area from 0.22 to 0.95 cm² plant⁻¹ compared with *F. solani* alone. Salicylic acid also improved several growth parameters in pathogen-inoculated plants, although its effect varied according to the *Fusarium* species and the measured trait.

Overall, the results demonstrate that infection with *F. proliferatum*, *F. oxysporum*, and *F. solani* adversely affected watermelon growth, with the magnitude of the reduction differing between the two cultivars. Both *A. niger* and salicylic acid alleviated some of the negative effects of *Fusarium* infection. However, *A. niger* generally produced a stronger recovery in root length, plant height, chlorophyll content, and leaf area, particularly in the Aseel cultivar,

suggesting that the effectiveness of the resistance-inducing treatments depended on both the watermelon genotype and the *Fusarium* species involved.

Table 3. Effect of different treatments on selected shoot and root growth parameters of the two watermelon cultivars

Treatment	Leaf area (cm ² plant ⁻¹)	Chlorophyll content (SPAD)	Aseel Plant height (cm plant ⁻¹)	Root length (cm plant ⁻¹)	Stem diameter (mm plant ⁻¹)	Leaf area (cm ² plant ⁻¹)	Chlorophyll content (SPAD)	Sugar Baby Plant height (cm plant ⁻¹)	Root length (cm plant ⁻¹)	Stem diameter (mm plant ⁻¹)
Control	0.96	6.4	19.77	11.9	0.12	1.92	13.08	20.8	14.23	0.15
Salicylic acid	0.96	8.77	20.33	12.7	0.12	2.22	14.4	23.6	15.07	0.15
<i>A. niger</i>	1.04	10.27	21.47	12.9	0.12	2.52	16.22	25	17.2	0.17
<i>F. proliferatum</i>	0.29	2.32	8.87	6.6	0.1	0.7	3.57	15.83	11.23	0.1
<i>F. oxysporum</i>	0.31	2.42	8.93	6.83	0.1	0.75	4	17.37	11.3	0.1
<i>F. solani</i>	0.22	1.75	8.37	6.47	0.12	0.69	3.33	14.57	10.27	0.1
<i>F. proliferatum</i> + <i>A. niger</i>	0.69	3.35	16.57	10.37	0.1	1.1	12.47	18.5	12.13	0.12
<i>F. oxysporum</i> + <i>A. niger</i>	0.89	4.32	17.4	11.27	0.1	1.45	6.78	18.7	12.6	0.13
<i>F. solani</i> + <i>A. niger</i>	0.95	6.12	18.3	11.27	0.1	1.01	5.68	17.5	11.6	0.12
<i>F. proliferatum</i> + Salicylic acid	0.64	2.72	12.33	9.57	0.1	1.65	6.9	19	13.27	0.12
<i>F. oxysporum</i> + Salicylic acid	0.52	3.12	13.7	10.12	0.1	1.66	8.1	19.27	13.9	0.13
<i>F. solani</i> + Salicylic acid	0.53	2.60	15.9	8.37	0.1	1.83	6.68	19.9	13.93	0.13
L.S.D	0.519	2.256	3.595	1.611	0.053	0.519	2.256	3.595	5.611	0.053

Similarly, higher chlorophyll content may reflect maintenance of the photosynthetic apparatus and reduced damage resulting from disease stress. Infection by vascular wilt fungi may disrupt water and nutrient transport, adversely affecting chlorophyll synthesis and vegetative growth. Therefore, maintaining relatively high chlorophyll content may contribute to the continued production of assimilates required for the development of new plant tissues (Al-Taie, 2014; Koo et al., 2020; Kasote et al., 2020; Ashraf et al., 2018).

Improved root growth may also play an important role in maintaining the uptake of water and mineral nutrients required for continued cell division and elongation. Likewise, improved stem diameter may be associated with enhanced structural growth and maintenance of vascular tissue efficiency under disease stress conditions (Pieterse et al., 2014; Backer et al., 2018).

4.3. Effect of Treatments on Fresh and Dry Biomass of Watermelon Seedlings

The treatments markedly affected the fresh and dry biomass of both watermelon cultivars. In Sugar Baby, *Aspergillus niger* alone recorded the highest shoot fresh weight (11.73 g), root fresh weight (1.71 g), and root dry weight (0.54 g), whereas salicylic acid alone produced the highest shoot dry weight (2.39 g). In contrast, inoculation with *Fusarium* species generally reduced plant biomass. The greatest reduction in Sugar Baby was associated with *F. proliferatum*, which reduced shoot fresh weight to 2.38 g compared with 7.67 g in the control. These reductions may be related to the adverse effects of *Fusarium* infection on root function, water and nutrient uptake, and consequently biomass accumulation.

Application of *A. niger* to *Fusarium*-inoculated plants considerably improved biomass accumulation. In Sugar Baby, shoot fresh weight increased from 2.38 to 7.39 g following *F. proliferatum* + *A. niger*, from 4.02 to 8.73 g following *F. oxysporum* + *A. niger*, and from 3.02 to 8.88 g following *F. solani* + *A. niger*. A similar response was observed in Aseel, where *A. niger* alone produced shoot and root fresh weights of 11.62 and 1.28 g and shoot and root dry weights of 2.27 and 0.52 g, respectively. Notably, the combination of *F. solani* + *A. niger* increased shoot fresh weight in Aseel to 11.29 g compared with 6.67 g in plants inoculated with *F. solani* alone.

These findings are consistent with Alwathnani and Perveen (2012), who reported that antagonistic fungal treatments, including *A. niger*, significantly increased plant fresh and dry weights under *Fusarium oxysporum* infection and that *A. niger* achieved 35.6% control of *Fusarium* wilt in tomato. Similarly, Attia et al. (2022) demonstrated the ability of plant growth-promoting fungi, including *A. niger*, to suppress *F. oxysporum* and enhance plant defense responses. The improved biomass observed in the present study may therefore result from reduced pathogen pressure together with growth-promoting and defense-stimulating effects associated with *A. niger*.

Salicylic acid also alleviated some of the biomass reductions caused by *Fusarium*, although its effectiveness varied according to the pathogen species and cultivar. This agrees with Zhu et al. (2022), who demonstrated that exogenous SA improved watermelon root performance following infection with *F. oxysporum* f. sp. *niveum*. In their study, the root fresh weight of SA-treated infected watermelon plants was approximately 2.5-fold higher than that of infected plants without SA treatment at 7 days post-inoculation, while disease incidence was also significantly reduced. Furthermore, Ling et al. (2009) reported that suppression of *Fusarium* wilt in watermelon by a bio-organic treatment was accompanied by increases of 73.3 and 66.7% in root fresh and dry weights, respectively, and 56.7 and 41.9% in above-ground fresh and dry biomass. Collectively, these previous findings support the present results, indicating that reducing *Fusarium*-associated stress can substantially restore biomass accumulation.

Overall, *A. niger* showed a more consistent positive effect on biomass in the present experiment, whereas the response to salicylic acid was more dependent on the *Fusarium* species and watermelon cultivar.

Table 4. Effect of different treatments on shoot and root fresh and dry weights of the two watermelon cultivars

Treatments	Aseel				Sugar Baby			
	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Control	6.85	0.91	0.94	0.38	7.67	0.8	2.03	0.42
Salicylic acid	10.97	0.99	1.03	0.45	9.3	0.88	2.39	0.42
<i>A. niger</i>	11.62	1.28	2.27	0.52	11.73	1.71	2.34	0.54
<i>F. proliferatum</i>	4.22	0.56	0.38	0.12	2.38	0.27	0.27	0.1
<i>F. oxysporum</i>	6.81	0.74	0.42	0.13	4.02	0.44	0.39	0.12
<i>F. solani</i>	6.67	0.31	0.69	0.13	3.02	0.31	0.58	0.12
<i>F. proliferatum</i> + <i>A. niger</i>	7.95	0.87	1.15	0.28	7.39	0.42	0.98	0.33
<i>F. oxysporum</i> + <i>A. niger</i>	8.06	0.6	1.19	0.25	8.73	0.79	1.49	0.27
<i>F. solani</i> + <i>A. niger</i>	11.29	1.22	1.27	0.17	8.88	0.71	1.69	0.19
<i>F. proliferatum</i> + Salicylic acid	6.56	0.97	1.69	0.25	6.76	0.21	0.54	0.15
<i>F. oxysporum</i> + Salicylic acid	5.77	0.65	1.87	0.22	7.17	0.91	1.98	0.15
<i>F. solani</i> + Salicylic acid	8.22	0.94	0.98	0.42	7.57	0.91	0.96	0.13
L.S.D	1.385	0.941	1.619	0.372	1.401	0.914	1.572	0.354

3.5 Effect of Treatments on Peroxidase Activity

The results (Figure 1) showed that peroxidase (POD) activity varied among the treatments and between the two watermelon cultivars. The highest activities were generally recorded when *Fusarium* infection was combined with salicylic acid or *Aspergillus niger*. In Sugar Baby, the highest POD activity was observed with *F. oxysporum* + SA (0.82) μmol tetra-guaiacol $\text{min}^{-1} \text{g}^{-1}$ FW, followed by *F. oxysporum* + *A. niger* (0.80) μmol tetra-guaiacol $\text{min}^{-1} \text{g}^{-1}$ FW. In Aseel, the highest activity was recorded with *F. proliferatum* + *A. niger* (0.79) μmol tetra-guaiacol $\text{min}^{-1} \text{g}^{-1}$ FW, followed by *F. oxysporum* + SA (0.76) μmol tetra-guaiacol $\text{min}^{-1} \text{g}^{-1}$ FW.

The increased POD activity in the combined treatments may indicate activation or priming of plant defense responses. Salicylic acid has been shown to enhance resistance of watermelon roots to *F. oxysporum* and to stimulate defense-related enzyme activity, including POD (Zhu et al., 2022). Peroxidases are involved in reactive oxygen species regulation, oxidation of phenolic compounds, lignification, and strengthening of cell walls, which can contribute to restricting pathogen invasion (Almagro et al., 2009).

The enhanced POD activity observed in treatments containing *A. niger* may also reflect stimulation of host defense mechanisms. Beneficial fungi, including *A. niger*, have been reported to suppress *Fusarium* wilt and enhance defense-related responses in infected plants (Attia et al., 2022). Overall, the results suggest that SA and *A. niger* enhanced biochemical defense responses against *Fusarium*, although the magnitude of the response depended on the cultivar and pathogen species.

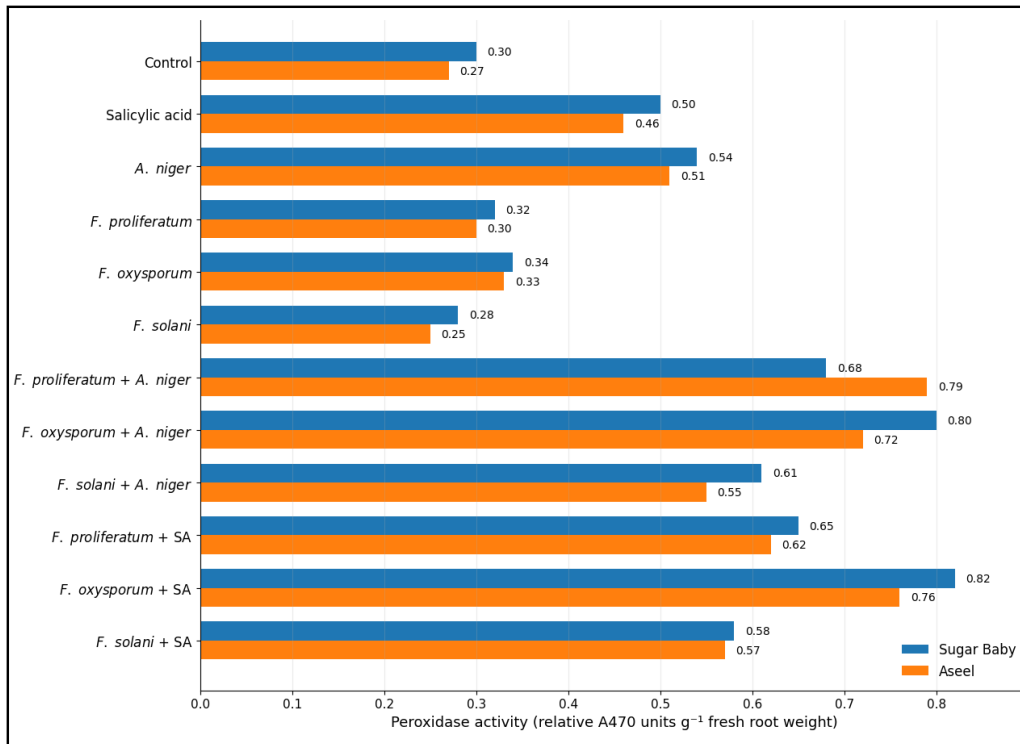


Figure 1. Effect of different treatments on peroxidase activity in watermelon roots

4- Conclusion

The results of this study demonstrate that salicylic acid and *Aspergillus niger*, when applied as resistance-inducing treatments, have the potential to enhance watermelon defense responses, improve plant growth, and reduce Fusarium-associated wilt incidence under greenhouse conditions. Their effectiveness varied according to the watermelon cultivar and Fusarium species involved. These findings suggest that salicylic acid and *A. niger* may represent promising components of integrated Fusarium-associated wilt management strategies. However, further field studies are required to confirm their effectiveness under natural conditions and to evaluate their effects on yield, fruit quality, and economic feasibility.

References

- Almagro, L., Gómez Ros, L. V., Belchi-Navarro, S., Bru, R., Ros Barceló, A., & Pedreño, M. A. (2009). Class III peroxidases in plant defence reactions. *Journal of Experimental Botany*, 60(2), 377–390.
- Al-Rawi, K. M., & Khalaf Allah, A. M. (1980). *Design and analysis of agricultural experiments*. Ministry of Higher Education and Scientific Research, Dar Al-Kutub Foundation for Printing and Publishing, University of Baghdad. 488 pp.
- Al-Taie, A. H. F. (2014). *Effect of some Aspergillus spp. and Trichoderma hamatum on the growth of cucumber (Cucumis sativus) grown in alternative culture media* [Doctoral dissertation, College of Agriculture, University of Kufa].
- Alwathnani, H. A., & Perveen, K. (2012). Biological control of Fusarium wilt of tomato by antagonist fungi and cyanobacteria. *African Journal of Biotechnology*, 11(5), 1100–1105. <https://doi.org/10.5897/AJB11.3361>

- Ashraf, N., Basu, S., Narula, K., Ghosh, S., Tayal, R., Gangisetty, N., Biswas, S., Aggarwal, P. R., Chakraborty, N., & Chakraborty, S. (2018). Integrative network analyses of wilt transcriptome in chickpea reveal genotype-dependent regulatory hubs in immunity and susceptibility. *Scientific Reports*, 8, 6528.
- Attia, M. S., Abdelaziz, A. M., Al-Askar, A. A., Arishi, A. A., Abdelhakim, A. M., & Hashem, A. H. (2022). Plant growth-promoting fungi as biocontrol tool against Fusarium wilt disease of tomato plant. *Journal of Fungi*, 8(8), 775.
- Backer, R., Rokem, J. S., Ilangumaran, G., et al. (2018). Plant growth-promoting rhizobacteria: Context, mechanisms of action, and road map to commercialization. *Frontiers in Plant Science*, 9, 1473. <https://doi.org/10.3389/fpls.2018.01473>
- Booth, C. (1971). *The genus Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England. 237 pp.
- Chance, B., & Maehly, A. C. (1955). Assay of catalases and peroxidases. *Methods in Enzymology*, 2, 764–775. [https://doi.org/10.1016/S0076-6879\(55\)02300-8](https://doi.org/10.1016/S0076-6879(55)02300-8)
- Dauda, N., Ugwuagu, S. N., Ishieze, P. U., Ugwuoke, K., Osadebe, V. O., Adewuyi, S. O., & Ukwu, U. N. (2024). Agronomic and disease responses of three watermelon (*Citrullus lanatus* L.) varieties to fungicide spraying regimes in a tropical environment. *Journal of Applied Biology & Biotechnology*, 12(3), 214–222. <https://doi.org/10.7324/JABB.2024.163372>
- du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae*, 196, 3–14. <https://doi.org/10.1016/j.scienta.2015.09.021>
- Everts, K. L., & Himmelstein, J. C. (2015). Fusarium wilt of watermelon: Towards sustainable management of a re-emerging plant disease. *Crop Protection*, 73, 93–99. <https://doi.org/10.1016/j.cropro.2015.02.019>
- Kasote, D. M., Sinha, R., Kaur, R., Kumar, R., Wechter, W. P., & Singh, R. (2020). Metabolomic and phytohormonal investigation of watermelon genotypes differing in resistance to *Fusarium* wilt. *Plant Physiology and Biochemistry*, 156, 492–501. <https://doi.org/10.1016/j.plaphy.2020.09.036>
- Koo, Y. M., Heo, A. Y., & Choi, H. W. (2020). Salicylic acid as a safe plant protector and growth regulator. *The Plant Pathology Journal*, 36(1), 1–10. <https://doi.org/10.5423/PPJ.RW.12.2019.0295>
- Ling, N., Wang, Q. J., Yang, X. M., Xu, Y. C., Huang, Q. W., & Shen, Q. R. (2009). Control of Fusarium wilt of watermelon by nursery application of bio-organic fertilizer. *Journal of Plant Nutrition and Fertilizers*, 15(5), 1136–1141. <https://doi.org/10.11674/zwyf.2009.0522>
- Lotfi, A. L. S. F. (1986). *Effect of nitrogen forms and calcium levels in nutrient solutions on the growth and yield of tomato plants* [Master's thesis, College of Agriculture, University of Baghdad, Iraq].
- Matroud, A. N. A. (2015). *Integrated management of charcoal rot disease of sunflower caused by Macrophomina phaseolina (Tassi) Goid.* [Ph.D. dissertation, Department of Plant Protection, College of Agriculture, University of Kufa, Iraq].
- Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., & Bakker, P. A. H. M. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology*, 52, 347–375. <https://doi.org/10.1146/annurev-phyto-082712-102340>
- Rahman, M. Z., Ahmad, K., Bashir Kutawa, A., Siddiqui, Y., Saad, N., Geok Hun, T., Hata, E. M., & Hossain, M. I. (2021). Biology, diversity, detection and management of *Fusarium*

oxysporum f. sp. *niveum* causing vascular wilt disease of watermelon (*Citrullus lanatus*): A review. *Agronomy*, 11(7), 1310. <https://doi.org/10.3390/agronomy11071310>

- Tawajin, A. M. M. (1979). *Greenhouse environment*. University of Basrah Press, pp. 571–573.
- Wang, Z., Jia, C., Li, J., Huang, S., Xu, B., & Jin, Z. (2015). Activation of salicylic acid metabolism and signal transduction can enhance resistance to *Fusarium* wilt in banana (*Musa acuminata* L. AAA group, cv. Cavendish). *Functional & Integrative Genomics*, 15, 47–62.
- White T. J., Bruns T. D., Lee S., Taylor J. W. (1990). "Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics," in PCR Protocols: a Guide to Methods and Applications, eds Innis M. A., Gelfand D. H., Sninsky J., White T. J. (California, CA: San Diego, Academic), 315–322. 10.1016/b978-0-12-372180-8.50042-1
- Yang, T., Zhang, P., Pan, J., Amanullah, S., Luan, F., Han, W., Liu, H., & Wang, X. (2022). Genome-wide analysis of the peroxidase gene family and verification of lignin synthesis-related genes in watermelon. *International Journal of Molecular Sciences*, 23(2), 642. <https://doi.org/10.3390/ijms23020642>
- Zhang, Y., & Li, X. (2019). Salicylic acid: Biosynthesis, perception, and contributions to plant immunity. *Current Opinion in Plant Biology*, 50, 29–36.
- Zhu, F., Fang, Y., Wang, Z., Wang, P., Yang, K., Xiao, L., & Wang, R. (2022). Salicylic acid remodeling of the rhizosphere microbiome induces watermelon root resistance against *Fusarium oxysporum* f. sp. *niveum* infection. *Frontiers in Microbiology*, 13, 1015038.