



Eco-friendly management of *Fusarium* wilt in tomato using *Salvia officinalis* methanolic extract: in vitro, in vivo, and molecular docking approaches

Mahmoud A. Gaber¹ · Sarah El-Messeiry² · Rania El-Tanbouly³ · Amany Assem Elkashef⁴ · Said I. Behiry⁵ · Sayed S. Aboshosha¹ · Helmy A. Aamer⁶

Received: 20 December 2024 / Accepted: 22 March 2025 / Published online: 14 April 2025
© The Author(s) 2025

Abstract

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici*, threatens global tomato production, with losses reaching 80%. Although chemical fungicides are effective, their prolonged use risks resistant strains, reduces soil biodiversity, and causes environmental damage, highlighting the urgent need for ecofriendly alternatives. This study investigated the viability of *Salvia officinalis* (sage) methanolic extract as a biocontrol agent against *Fusarium* wilt (FW), employing a comprehensive approach that incorporates in vitro, in vivo, and molecular docking techniques. Four distinct isolates of *F. oxysporum* were identified through molecular techniques, and their virulence was assessed by examining the presence of tomatinase genes. The antifungal properties of *S. officinalis* extract were found to be compelling, with a total phenolic content of 64.15 mg GAE/g and a remarkable antioxidant activity of 97.04%. In laboratory tests, *S. officinalis* exhibited potent antifungal activity, inhibiting mycelial growth by between 52.00% and 88.67% at a concentration of 20 mg/ml. Additionally, in vivo experiments demonstrated a significant reduction in disease severity in treated tomato plants. Molecular docking analyses revealed strong binding affinities between key phytochemicals in the extract and target receptors such as tomatinase, highlighting the potential of the extract as a sustainable and effective alternative to chemical fungicides for managing FW in tomato crops.

Keywords Tomato wilt · *Fusarium oxysporum* · GC–MS · Eco-friendly · Tomatinase gene · Virulence

✉ Mahmoud A. Gaber
mahmoudgaber@alexu.edu.eg

✉ Rania El-Tanbouly
ran.eltanbouly@alexu.edu.eg

Sarah El-Messeiry
sarah.elmesseiry@alexu.edu.eg

Amany Assem Elkashef
amany.assem_pg@alexu.edu.eg

Said I. Behiry
said.behiry@alexu.edu.eg

Sayed S. Aboshosha
sayed.aboshosha@alexu.edu.eg

Helmy A. Aamer
helmy.amer@alexu.edu.eg

¹ Department of Plant Pathology, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria 21545, Egypt

² Department of Genetics, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria 21545, Egypt

³ Department of Floriculture, Ornamental Horticulture, and Landscape Gardening, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria 21545, Egypt

⁴ Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt

⁵ Agricultural Botany Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria 21531, Egypt

⁶ Department of Pesticide Chemistry and Technology, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria 21545, Egypt

Introduction

Fusarium wilt, caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *lycopersici* (FOL), represents one of the most formidable challenges to tomato cultivation globally (Gaber et al. 2022). This disease leads to significant economic losses due to its prevalence and severe impact on crop yield and quality. The symptoms of Fusarium wilt typically include yellowing of leaves, wilting, and eventual plant collapse, which can devastate entire fields if not managed effectively (Gordon 2017). The widespread nature of this pathogen and its ability to persist in soil for extended periods complicate management efforts, necessitating innovative approaches for disease control. Historically, chemical fungicides have been the primary means of managing fusarium wilt (Egel and Martyn 2007). However, the extensive use of these chemicals has raised concerns regarding their environmental impact, potential toxicity to non-target organisms, and the development of resistant strains of pathogens (Abdelaziz et al. 2024; Himabindu and Kumar 2021). *Salvia officinalis*, commonly known as sage, has garnered attention for its antifungal properties, particularly against various fungal pathogens that affect plants. Research has demonstrated that the essential oil and extracts of *S. officinalis* exhibit significant antifungal activity against species such as *Candida albicans*, *Candida glabrata*, and *Fusarium graminearum* (De Oliveira et al. 2019). These antifungal effects are attributed to the presence of bioactive compounds, including 1,8-cineole, camphor, and thujone, which disrupt fungal cell membranes and inhibit growth (Ghorbani and Esmaeilzadeh 2017; Jazo et al. 2023; Khedher et al. 2017).

Recent studies have highlighted the potential of *Salvia officinalis* extracts in agricultural applications, particularly against phytopathogenic fungi. The essential oils derived from sage have demonstrated significant antifungal activity against a variety of pathogens, including *Fusarium oxysporum*, *Botrytis cinerea*, and *Alternaria solani*, which are known to cause severe crop losses (Porte et al. 2013; Zaccardelli et al. 2020). These extracts contain bioactive compounds such as rosmarinic acid and carnosic acid, which not only exhibit strong antioxidant properties but also increase plant resistance to diseases (Kursa et al. 2022; Youssef et al. 2021). Furthermore, studies have shown that sage essential oil can inhibit the growth of bacterial pathogens such as *Escherichia coli*, thereby contributing to improved food safety in agricultural products (De Oliveira et al. 2019; Lahlou et al. 2023). Biocontrol methods utilizing natural plant extracts have emerged as promising alternatives, offering the dual benefit of disease management while reducing the reliance on synthetic chemicals. Studies have demonstrated that extracts from *S. officinalis* exhibit potent

antifungal activity against several fungal species, making it a potential biocontrol agent against FW (Abdelkhalek et al. 2022; Kursa et al. 2022; Youssef et al. 2021). Research indicates that these extracts can significantly reduce disease severity when applied to infected tomato plants, suggesting their practical application in agricultural settings (Krif et al. 2024; Kumar et al. 2024).

Molecular docking studies have opened new avenues for understanding the interactions between phytochemicals and fungal targets at the molecular level. These studies can identify specific binding sites and elucidate the mechanisms by which these compounds exert their antifungal effects (Cheng et al. 2023; Singh et al. 2023).

This research aims to use molecular docking methods along with traditional biological tests by identifying and characterizing the phytochemical compounds in a methanolic extract of *Salvia officinalis* using gas chromatography–mass spectrometry (GC–MS). The antifungal properties of the isolates of the pathogen *Fusarium oxysporum* f. sp. *lycopersici* (FOL) were studied through a comprehensive approach that included in vitro assays, in vivo trials on tomato plants, and molecular docking analyses. We seek to enhance our understanding of *Salvia officinalis* as a biocontrol agent and promote ecofriendly agricultural practices that align with global sustainability goals by providing a sustainable alternative to chemical fungicides, reducing environmental pollution and the risks associated with chemical residues in food.

Materials and methods

Fungal isolates used in the study

Fungal isolates were collected from infected tomato plants and rhizosphere soil exhibiting wilt symptoms across various locations across different regions in Alexandria, Egypt. The isolation process involved collecting root and soil samples from symptomatic plants, followed by isolation by sterilization and culture on potato dextrose agar (PDA) plates supplemented with aureomycin hydrochloride as an antibacterial agent and incubation at 27 °C for 7 days. These isolates were subsequently characterized morphologically and molecularly to confirm their identity as *Fusarium oxysporum* f. sp. *lycopersici* (FOL) (López-Zapata et al. 2021; Srinivas et al. 2019).

DNA isolation, PCR amplification, sequence analysis, and phylogenetic tree construction

To identify the isolates at the molecular level, we employed a polymerase chain reaction (PCR) technique utilizing

internal transcribed spacer (ITS) regions, specifically the primers ITS1 and ITS4 (White et al. 1990). Additionally, we amplified elongation factor-1 alpha (EF-1 α) via the primers EF1-H (5'-ATGGGTAAGGAAGACAAGAC-3') and EF2-T (5'-GGAAGTACCAGTGATCATGTT-3') (O'donnell et al. 1998), as well as the beta-tubulin (TUB2) gene via the primers T1 (5'-AACATGCGTGAGATTGT AAGT-3') and T22 (5'-TCTGGATGTTGGGAATCC-3') (O'donnell and Cigelnik 1997). Fungal isolates were cultured on potato dextrose agar (PDA), and genomic DNA was extracted via a standard phenol–chloroform method as described previously (Green and Sambrook 2012). PCR amplification was performed in a total volume of 25 μ L, containing 1 ng of genomic DNA, 1X PCR buffer, 0.2 mM dNTPs, 0.2 μ M of each primer (ITS1: 5'-TCCGTAGGTG AACCTGCG-3' and ITS4: 5'-TCCTCCGCTTATTGATAT GC-3'), 1.5 mM MgCl₂, and 1 U of Dream Taq polymerase (Thermo Fisher Scientific, U.S.A.). The thermal cycling conditions included initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s for ITS, 60 °C for 2 min for *EF-1 α* and *TUB2*, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. The amplified products were analyzed via electrophoresis on a 2% agarose gel in TBE (0.5X) stained with 10 μ g/ml ethidium bromide, revealing bands corresponding to the expected size of approximately 600 bp. The PCR products were purified via a PCR product purification kit (Thermo Fisher Scientific, U.S.A.) and subsequently sequenced via the Sanger sequencing method. The acquired sequences were compared with those available in the GenBank database via the BLAST algorithm to accurately identify the fungal species. Sequence alignment was performed via the Clustal Omega program, followed by the construction of a phylogenetic tree via the maximum-likelihood method. Phylogenetic analysis was conducted via MEGA 5 software. This analysis of genetic sequences provided valuable insights into the diversity of fungi and facilitated the identification of distinct fungal species (Gaber et al. 2019; Shakam and Gaber 2024).

Molecular validation of the genes encoding Tomatinase

The tomatinase genes of the FOL isolates were assessed via polymerase chain reaction (PCR) to target specific tomatinase genes, which were designated tom-1, tom-2, tom-3, tom-4, and tom-5 (Supplementary Table 1) as described by (Pareja-Jaime et al. 2008a). The PCR mixture was set up in a total volume of 25 μ L, which included 1 ng of genomic DNA, 0.2 μ M of each primer, 1X PCR buffer, 0.2 mM dNTPs, 1.5 mM MgCl₂, 1 U of Taq polymerase (Thermo Fisher Scientific), and sterile distilled water (diH₂O). The

amplification process was performed via 35 cycles of denaturation at 94 °C for one minute, annealing at 58 °C for one minute, and extension at 72 °C for one minute, followed by a final extension step at 72 °C for ten minutes. The resulting PCR products were analyzed via gel electrophoresis on a 2% agarose gel (0.5X) TBE run at 100–150 V for 20–55 min to separate the amplified fragments, which were subsequently stained with Red Safe dye to visualize the bands. A DNA ladder (Biohelx, Korea) with increments of 100 base pairs was utilized as a standard for measuring molecular sizes. Photographs of the gel were captured via a UV transilluminator to document the presence and size of the amplified tomatinase gene fragments.

Plant material extraction

Preparation of *S. officinalis* extract

The aerial parts of *S. officinalis* were completely air-dried at room temperature. Then, 10 g of powdered *S. officinalis* was macerated in 100 ml of 80% methanol for 48 h at room temperature while being constantly shaken. Whatman No. 1 filter paper was used to separate the extract, which was subsequently vacuum-dried at 40 °C and freeze-dried. The extract powder was stored at -20 °C until further use.

Phytochemical screening

Total phenolic content (TPC)

The total phenolic content was assessed utilizing the Folin-Ciocalteu technique with slight modifications (Singleton and Rossi 1965). A total of 0.5 ml of extract solution was combined with 2 ml of diluted Folin-Ciocalteu reagent (1:10 in deionized water) and incubated at ambient temperature (26 $\pm$ 2 °C) for 3 min. Subsequently, 1 ml of 7.5% sodium carbonate solution was added, and the mixture was incubated at 40 °C for 30 min. The absorbance was quantified at 765 nm via a UV–Vis spectrophotometer, with deionized water used as the reference blank. The total phenolic content was determined via a gallic acid calibration curve and represented as milligrams of gallic acid equivalent (GAE) per gram of dried extract.

Total flavonoid content

The flavonoid concentration was quantified via the aluminum chloride colorimetric method (Woisky and Salatino 1998). One milliliter of extract solution in methanol was combined with 2 ml of 2% aluminum chloride in methanol. The mixture was agitated vigorously and incubated at

ambient temperature (25 ± 2 °C) in the absence of light for 1 h. The absorbance was assessed at 420 nm, and the flavonoid concentration was determined utilizing a quercetin calibration curve, given as milligrams of quercetin equivalents (QEs) per gram of dried extract.

Antioxidant activity

DPPH radical scavenging activity The DPPH radical scavenging activity was evaluated using the method established by (Bakar et al. 2009). Solutions were produced at concentrations between 2.4 and 175 µg/ml. A 40 µg/ml DPPH solution in methanol was utilized. One hundred microliters of extract (or ascorbic acid as a positive control) was combined in triplicate with 1.2 ml of DPPH solution and incubated in the dark at ambient temperature for 30 min. The absorbance was recorded at 517 nm, and the radical scavenging activity was determined using the following formula:

$$\text{Radical Scavenging Activity (\%)} = \frac{\left(\frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \right) \times 100}{\text{Control Absorbance}}$$

Total antioxidant capacity The total antioxidant capacity was assessed via the phosphomolybdenum test (Prieto et al. 1999). A 400 µL extract solution (or ascorbic acid standard, 20–200 µg/ml) was combined with 2.8 mL of phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The mixture was incubated at 95 °C for 90 min in darkness and subsequently cooled to room temperature. The absorbance was quantified at 695 nm, and the total antioxidant capacity was calculated as the ascorbic acid equivalent (AAE) per gram of dried extract.

Gas Chromatography–Mass Spectrometry (GC–MS) analysis

The methanolic extract was subjected to gas chromatography–mass spectrometry (GC–MS) analysis to identify the volatile compounds present in the extract. We utilized a Trace GC-TSQ mass spectrometer produced by Thermo Scientific in Austin, Texas, USA. The mass spectrometer was outfitted with a TG-5MS column that was 30 m in length, 0.25 mm in diameter, and 0.25 µm thick. The column temperature was initially set at 30 degrees Celsius and maintained for two minutes. The temperature was subsequently consistently elevated to 300 degrees Celsius at a pace of 30 degrees Celsius per minute. The overall runtime was forty-five minutes. Helium served as the carrier gas, with a flow

rate of one milliliter per minute. Mass spectra were acquired in electron impact (EI) mode at a potential energy of 70 eV, covering a mass range of 50–550 m/z. A Thermo Xcalibur was used for data analysis and gathering. To aid in the identification of compounds, the WILEY 09 and NIST 14 mass spectrum databases were compared with the identified compounds (Aamer et al. 2023).

In vitro antifungal activity of *Salvia officinalis* extract

Mycelial growth inhibition

The antifungal activity was evaluated via the poisoned food method (Grover and Moore 1962). *Salvia officinalis* extract was integrated into PDA at doses of 2.5, 5, 10, and 20 mg/ml. A 5 mm mycelial agar disc from an 8-day-old *F. oxysporum* colony was positioned at the center of each plate. Control plates devoid of extract (DMSO only) were made for each isolate. The plates were incubated in darkness at 25 °C, and radial growth was assessed when the control plates achieved complete coverage. The percentage of mycelial growth inhibition was determined via the following formula:

$$\text{Percentage growth Inhibition (\%)} = \frac{\left(\frac{\text{Growth in Control} - \text{Growth in Treatment}}{\text{Growth in Control}} \right) \times 100}{\text{Growth in Control}}$$

Conidia germination inhibition

Conidial germination was assessed by generating a mixture comprising a conidial suspension, Czapek Dox broth, and plant extract at doses of 2.5, 5, 10, and 20 mg/ml. The mixtures were maintained at 28 °C for 24 h (Rongai et al. 2012). Microscopic examination of conidial germination was conducted, and the percentage of germination was averaged from triplicate experiments.

Greenhouse experiment

Tomato seeds (*Solanum lycopersicum* L. Cv. 023) were surface sterilized with 1% sodium hypochlorite for 2 min and subsequently rinsed with sterile distilled water. Ten seeds were planted in each container in a completely randomized pattern.

Three weeks post-sowing, the methanolic extract of *S. officinalis* (20 mg/ml, 50 ml per plant) was applied to the rhizosphere. Additionally, the commercial fungicide Topsin-M (70% WP thiophanate-methyl) was used at the recommended rate as a positive control for comparison. Microconidia of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) were produced as a suspension (1×10^6 spores/ml

in 0.1% Tween 80) and applied to roots 48 h after extract/ or fungicide treatment. Untreated plants served as negative controls.

Qualitative disease severity was assessed on the plant shoots four weeks post inoculation via a 0–4 scale, as described by (Song et al. 2004). The scale was as follows: • 0: No infection. • 1: Mild chlorosis, stunting, or wilting • 2: Moderate chlorosis, stunting, or wilting • 3: Severe chlorosis, stunting, or wilting • 4: Mortality. The disease severity percentage of the disease was subsequently calculated via the following formula:

$$\text{Disease severity}\% = \frac{\sum \left(\frac{\text{number of plants} \times \text{number of degree in symptom}}{\text{Total number of plants}} \right)}{\times \text{maximum degree in symptom}} \times 100$$

Additionally, disease prevention measures were performed via the following formula according to Song et al. (2004). The formula used:

$$\text{Disease preventive \%} = \frac{\text{disease severity (control)} - \text{disease severity (treatment)}}{\text{disease severity (control)}} \times 100$$

Molecular docking analysis

The active ingredients of the methanolic extract were subjected to active site molecular docking simulation against tomatinase, an endo-1,4-beta-glycosidase receptor. The 3D structure of the receptor was downloaded from the Alpha-Fold Protein Structure (<https://alphafold.ebi.ac.uk/>) database; subsequent protein pretreatments, including removing water molecules and heteroatoms as well as protonation and minimizing its energy via Molecular Operating Environment (MOE 2014.10, Chemical Computing Group, Montreal, QC, Canada) software, were performed. The 3D structures of the active ingredients of the methanolic extract were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). They were also prepared by adding polar hydrogens and partial charges and minimizing their energy before they entered the molecular docking simulation. Finally, the docking process and visualization of the ligand–protein interactions were performed via MOE software.

Statistical analysis

All experiments were carried out in triplicate, and the mean ± standard deviation (SD) was used to express the results. One-way analysis of variance (ANOVA) and Tukey's post hoc test for multiple comparisons were used to assess the statistical significance of the differences in means.

SAS software (SAS Institute Inc., Cary, NC) was used for statistical analysis, and significance thresholds were set at $p < 0.05$.

Results

Molecular identification of FOL isolates

Four fungal isolates were consistently obtained from the root tissues of tomato plants exhibiting wilt and from the soil rhizosphere and cultivated on PDA. The isolates were initially classified at the genus level according to their morphological traits. The colonies of *Fusarium* FOL exhibited a white to pink hue, with conidia identified as microconidia alongside chlamydospores, as viewed under a light microscope. The phylogenetic relationships among the four *Fusarium oxysporum* f. sp. *lycopersici* isolates (FOL501, FOL502, FOL503, and FOL504) were analyzed via three distinct genes: the ITS region, elongation factor-1 alpha (EF-1α), and beta-tubulin (TUB2). Maximum likelihood (ML) phylogenetic trees were constructed for each gene to assess the evolutionary clustering of these isolates (Fig. 1). The phylogenetic tree was built using the MEGA program version 11. The reliability of the assumed phylogeny was considered through 1,000 bootstrap replicates. The nucleotide sequence, along with annotations, has been uploaded to the GenBank database under the accession numbers PQ516909, PQ516910, PQ516911, and PQ516912 for the ITS region; PV199627, PV199628, PV199629 and PV199630 for the *EF-1α* gene; and PV199631, PV199632, PV199633 and PV199634 for the TUB2 gene. The nucleotide sequences of the four isolates exhibited comprehensive sequence coverage and congruence with other related species in GenBank, notably the South Korean *Fusarium oxysporum* f. sp. *lycopersici* strain (Accession No. DQ452454.1), and we used *Fusarium solani* from the group rooted as depicted in Fig. 1.

The ITS-based phylogenetic tree (Fig. 1a) placed FOL501, FOL502, FOL503, and FOL504 within a strongly supported clade along with other known *F. oxysporum* f. sp. *lycopersici* isolates, confirming their identity. This clade was distinct from other formae speciales of *F. oxysporum*, such as *F. oxysporum* f. sp. *lactucae* and *F. oxysporum* f. sp. *conglutinans*.

Phylogenetic analysis based on EF-1α (Fig. 1b) further supported the clustering of FOL501–FOL504 within *F. oxysporum* f. sp. *lycopersici*, with a bootstrap value of 88, indicating a close evolutionary relationship among these tomato pathogenic isolates.

Similarly, the TUB2-based phylogenetic tree (Fig. 1c) showed a consistent clustering pattern, with FOL501, FOL502, FOL503, and FOL504 grouping within *F.*

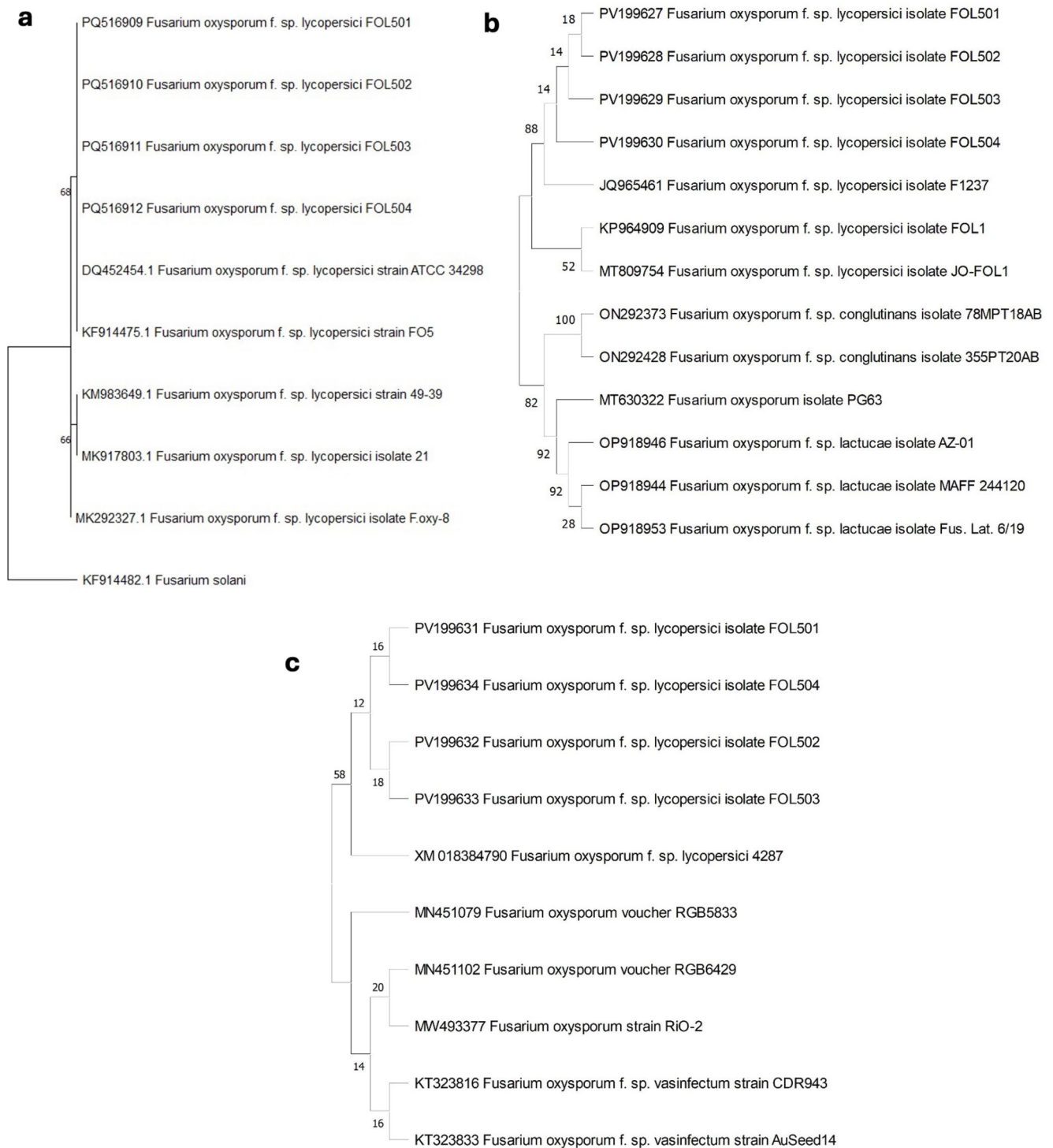


Fig. 1 Phylogenetic tree of the four fungal isolates FOL501, FOL502, FOL503, and FOL504 constructed on the basis of three distinct identification genes: **(a)** ITS region sequences, **(b)** elongation factor-1 alpha (*EF-1α*) gene partial sequences, and **(c)** beta-tubulin (*TUB2*) gene par-

tial sequences. The tree was generated via the maximum likelihood method, with bootstrap values based on 1,000 replications indicated at the branch nodes

Table 1 The total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (DPPH assay) and total antioxidant capacity (TAC) of the *S. officinalis* extract were measured

TPC (mg GAE/g Dry Extract)	TFC (mg QE/g Dry Extract)	DPPH (IC ₅₀)	TAC
64.15 ± 1.59	54.04 ± 1.65	97.04 ± 0.87	108.10 ± 1.34

Table 2 Screening of tomatinase (*Tom*) genes

Isolate	Tom1 ^b	Tom2 ^c	Tom3 ^d	Tom4 ^e	Tom5 ^f
FOL 501	-	+	+	+	+
FOL 502	-	+	+	+	+
FOL 503	+	+	+	-	+
FOL 504	+	+	+	+	+

+, present; -, absent; a, presence or absence of 600-bp amplicon; b, presence or absence of 550-bp amplicon; c, presence or absence of 600-bp amplicon; d, presence or absence of 700-bp amplicon; e, presence or absence of 600-bp amplicon

oxysporum f. sp. *lycopersici*. However, the bootstrap value for this clade was slightly lower (58), suggesting a relatively weaker but still significant phylogenetic signal from this marker.

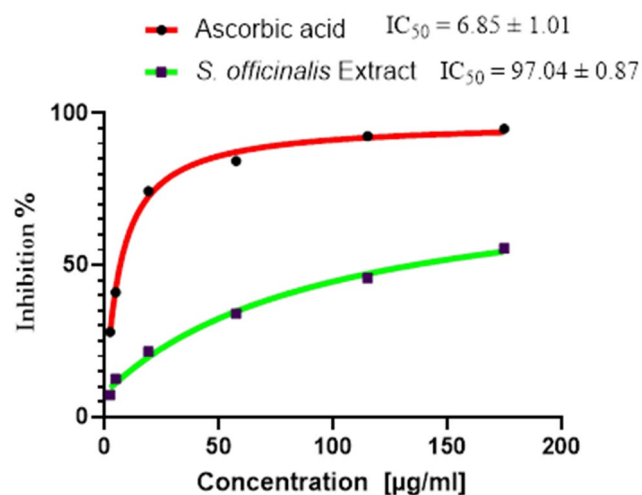
Molecular analysis of the genes encoding tomatinase

The four isolates of FOL Saccardo infecting tomatoes were screened for the presence of different tomatinase genes (*tom-1*, *tom-2*, *tom-3*, *tom-4* and *tom-5*). An amplicon length of ~600 bp was detected in all the isolates for the *tom-2*, *tom-3* and *tom-5* genes, whereas the *tom-4* gene, with an amplicon length of ~700 bp, was detected in only isolates except for FOL503. In contrast, the *tom-1* gene, with an amplicon of ~600 bp, was detected in two isolates (FOL503 and FOL504) (Table 1 and Supplementary Fig. S1).

In this study, five tomatinase genes (Tom1b, Tom2c, Tom3d, Tom4e, and Tom5f) were screened across four FOL isolates. The screening results revealed that FOL 501 and FOL 502 tested positive for Tom2c, Tom3d, Tom4e, and Tom5f but negative for Tom1b. In contrast, FOL 503 was positive for Tom1b, Tom2c, Tom3d, and Tom5f but negative for Tom4e. Notably, FOL 504 presented a complete set of tomatinase genes (Table 2). This pattern suggests that while some isolates possess a full complement of tomatinase genes, others may lack specific genes that could affect their virulence.

Phytochemical screening analysis of *S. officinalis* methanolic extract

The phytochemical content and antioxidant properties of the *S. officinalis* methanolic extract are summarized in Table 1. The total phenolic content (TPC) was determined to be 64.15 ± 1.59 mg GAE/g dry extract, whereas the total

**Fig. 2** DPPH IC₅₀ values of *S. officinalis* methanolic extract and ascorbic acid

flavonoid content (TFC) was measured at 54.04 ± 1.65 mg QE/g dry extract. The antioxidant activity, assessed via the DPPH assay, yielded an IC₅₀ value of 97.04 ± 0.87, indicating its ability to scavenge free radicals. The total antioxidant capacity (TAC) was recorded as 108.10 ± 1.34, highlighting the extract's robust antioxidant potential.

Figure 2 compares the DPPH antioxidant activity of ascorbic acid and the *S. officinalis* methanolic extract, illustrating their percentage inhibition across varying concentrations. Ascorbic acid has significantly greater activity, with an IC₅₀ value of 6.85 ± 1.01 µg/ml, indicating strong antioxidant potential. In contrast, the *S. officinalis* extract had a much higher IC₅₀ of 97.04 ± 0.87 µg/ml, reflecting a lower antioxidant efficiency than ascorbic acid.

GC–MS analysis of the *S. officinalis* methanolic extract

The analysis revealed a diverse range of compounds, predominantly terpenoids, fatty acids, and phenolic compounds. Among the terpenoids, eucalyptol (8.47%) and camphor (5.65%) were the most abundant, with notable contributions from caryophyllene (2.77%) and podocarpa-1,8,11,13-tetraen (7.62%). Fatty acids such as oleic acid (5.8% combined across two entries), pentadecanoic acid (5.64%), and octadecanoic acid (1.58%) were significant. Phenolic compounds such as 12-O-methylcarnosol (5.42% and 1.37%) and isocarnosol (3.63%) were also prominent. Additionally, carbohydrates included α-D-glucopyranoside and methyl groups (15.73%), and relatively small amounts of anthracene derivatives, diterpenoids, carotenoids, and gibberellins were detected (Fig. 3; Table 3).

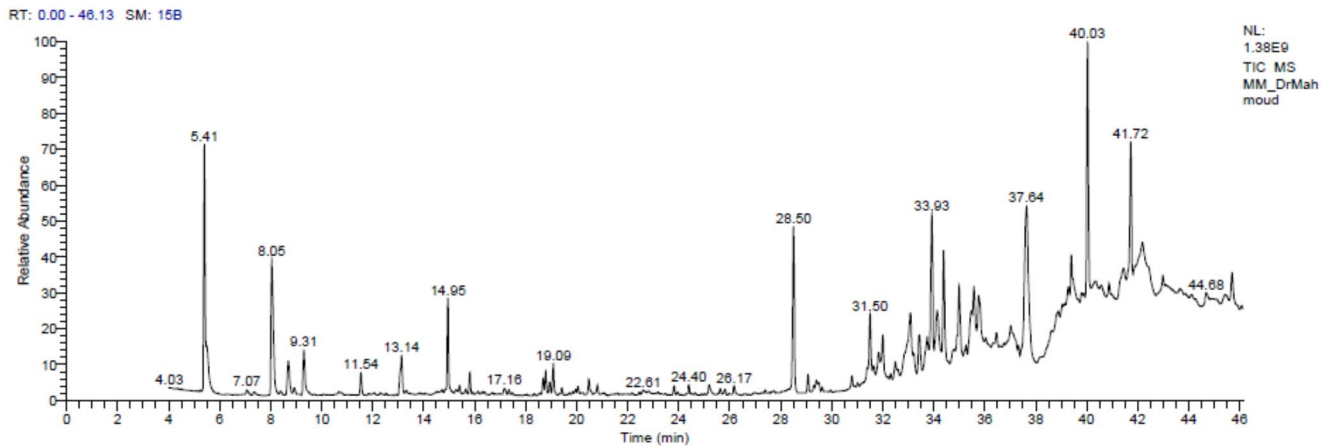


Fig. 3 GC–MS chromatogram of the *S. officinalis* methanolic extract

Antifungal activity of the *S. officinalis* methanolic extract

The data show the inhibitory effects of *S. officinalis* methanolic extract concentrations (2.5, 5, 10, and 20 mg/ml) on the mycelial growth and spore germination of four isolates (Fol 501, Fol 502, Fol 503, and Fol 504).

For mycelial growth inhibition, Fol 501 consistently demonstrated the greatest inhibition, ranging from 43.67% at 2.5 mg/ml to 88.67% at 20 mg/ml, significantly outperforming the other isolates. Fol 502 followed by moderate inhibition (35.33–71.00%), whereas Fol 503 and Fol 504 presented the lowest inhibition, with values ranging from 15.67 to 52.00% and 16.67–61.00%, respectively (Table 4).

In terms of spore germination, Fol 501 again presented the highest inhibition rates (34.15–84.51%), followed closely by Fol 503, particularly at relatively high concentrations (24.51–87.55%). Fol 502 demonstrated moderate spore germination inhibition (22.30–66.54%), whereas Fol 504 consistently presented the lowest inhibition rates, ranging from 4.33 to 51.99% (Table 4).

Greenhouse experiment

The severity of disease caused by four different isolates of *Fusarium oxysporum* (FOL 501, FOL 502, FOL 503, and FOL 504) was evaluated in tomato plants across three treatment groups: control (untreated), sage extract-treated, and fungicide-treated (thiophanate-methyl), as shown in Fig. 4a. The untreated control group presented the greatest degree of disease severity, with levels consistently ranging from 45 to 75%. This finding indicates the strong pathogenicity of the isolates in the absence of treatment, with the highest severity observed in the FOL 503 isolate and the lowest in FOL 501. In contrast, compared with the control treatment, the sage extract treatment significantly reduced disease severity,

even in the presence of the highly virulent FOL 503 isolate. This highlights the potential antifungal properties of sage extract. However, while the sage extract showed some efficacy, its performance was not as strong as that of the chemical fungicide treatment. Thiophanate-methyl treatment was the most effective, consistently reducing disease severity to less than 10% across all the isolates, demonstrating its superior ability to control FW.

The preventative effects of sage extract and a thiophanate-methyl chemical fungicide on FW were evaluated across four FOL isolates (Fig. 4b). Thiophanate-methyl consistently resulted in greater disease reduction than the sage extract in all the isolates tested. Although the sage extract demonstrated some preventative effects across all the isolates, its efficacy varied, with the greatest reduction against FOL 501 and the lowest against FOL 503. While thiophanate-methyl consistently achieved high levels of disease reduction, the sage extract also demonstrated relatively consistent preventative percentages across the different isolates. These results indicate that while sage extract has potential as a natural antifungal agent, thiophanate-methyl remains the most effective treatment for managing Fusarium wilt in tomatoes. However, the consistent performance of sage extract suggests its potential utility as an alternative or complementary treatment within eco-friendly or organic disease management strategies.

Molecular docking analysis

Using MOE software, the in silico docking simulation of the active ingredients of the rosemary methanolic extract was performed by selecting the first two active sites of the receptor and increasing the number of active ingredients to 60. The molecular docking scores represented the binding affinity between the active compounds and Tomatinase, an endo-1,4-beta-glycosidase receptor. The docking scores revealed

Table 3 Chemical composition of *Salvia officinalis* methanolic extract via GC–MS analysis

RT	Area %	Compound	Class
5.41	8.47	Eucalyptol	Terpenoids
8.05	5.65	Camphor	Terpenoids
8.69	1.41	Isoborneol	Terpenoids
9.31	1.63	α -Terpineol	Terpenoids
11.54	0.7	Bornyl acetate	Terpenoids
13.14	1.65	α -Terpinyl acetate	Terpenoids
14.95	2.77	Caryophyllene	Terpenoids
15.41	0.23	Aromadendrene	Terpenoids
15.82	0.64	α -Caryophyllene	Terpenoids
18.69	0.46	Spathulenol	Terpenoids
18.95	0.35	Caryophyllene oxide	Terpenoids
19.09	0.94	10-Undecenoic acid	Fatty Acids
19.42	1.3	Viridiflorol	Terpenoids
23.82	0.28	Caryophyllene oxide	Terpenoids
24.4	0.31	Fenretinide	Retinoids
25.19	0.34	Neophytadiene	Phytochemical
28.5	5.64	Pentadecanoic acid	Fatty Acids
29.4	0.49	Palmitic acid	Fatty Acids
29.51	0.28	Linolenic acid	Fatty Acids
30.79	0.45	Palmitelaidic acid	Fatty Acids
30.79	0.45	Phytol	Phytochemical
31.4	0.34	Linoelaidic acid	Fatty Acids
31.51	2.43	Oleic acid	Fatty Acids
31.65	0.44	Petroselinic acid	Fatty Acids
32.01	1.58	Octadecanoic acid	Fatty Acids
32.85	7.62	Podocarpa-1,8,11,13-tetraen	Diterpenoids
33.93	5.42	12-O-Methylcarnosol	Phenolic Compounds
34.39	3.63	Isocarnosol	Phenolic Compounds
35	2.98	9-Anthracenol	Anthracene
35.47	1.75	9,10-Dimethoxyanthro	Anthracene
35.76	1.85	Carnosol	Phenolic Compounds
37.02	0.75	Gibberellic acid	Gibberellins
37.64	15.73	α -D-Glucopyranoside, methyl	Carbohydrates
38.82	0.31	$\psi\psi$ -Carotene, 2-tetrahydro-1-dimethoxy	Carotenoids
39.05	3.37	Oleic acid	Fatty Acids
39.39	1.37	12-O-Methylcarnosol	Phenolic Compounds
42.18	1.46	9-Hexadecenoic acid	Fatty Acids

* RT retention time

significant variations in binding affinity among the active ingredients (Table 5).

For the extract, oleic acid was the ingredient with the lowest free energy of binding (-8.69 kcal/mol, Fig. 5a), followed by α -D-glucopyranoside (-8.35 kcal/mol, Fig. 5b) and then $\psi\psi$ -carotene, 2-tetrahydro-1-dimethoxy (-7.90 kcal/mol, Fig. 5c), which had H-bonding through its methoxy oxygen with HIS305(A).

Table 4 The antifungal effects of *S. officinalis* extract on the mycelial growth and spore germination of FOL isolates

Iso-lates code	Extract Concentration (mg/ml)*			
	2.5	5	10	20
Mycelial growth inhibition (%)				
Fol 501	43.67 $\pm$ 2.52 ^a	57.00 $\pm$ 2.00 ^a	76.67 $\pm$ 2.08 ^a	88.67 $\pm$ 3.06 ^a
Fol 502	35.33 $\pm$ 2.08 ^b	45.67 $\pm$ 1.53 ^b	54.33 $\pm$ 1.15 ^b	71.00 $\pm$ 2.00 ^b
Fol 503	15.67 $\pm$ 2.08 ^c	27.00 $\pm$ 2.00 ^c	38.33 $\pm$ 3.06 ^b	52.00 $\pm$ 1.00 ^b
Fol 504	16.67 $\pm$ 1.53 ^c	31.00 $\pm$ 2.65 ^c	49.00 $\pm$ 2.00 ^c	61.00 $\pm$ 2.00 ^c
Spore germination (%)				
Fol 501	34.15 $\pm$ 3.23 ^a	57.04 $\pm$ 3.40 ^a	71.13 $\pm$ 2.44 ^a	84.51 $\pm$ 2.66 ^a
Fol 502	22.30 $\pm$ 2.32 ^b	38.29 $\pm$ 3.41 ^c	56.51 $\pm$ 3.35 ^b	66.54 $\pm$ 2.95 ^b
Fol 503	24.51 $\pm$ 2.94 ^b	48.25 $\pm$ 2.94 ^b	71.60 $\pm$ 2.43 ^a	87.55 $\pm$ 3.57 ^a
Fol 504	4.33 $\pm$ 3.31 ^c	14.44 $\pm$ 2.87 ^c	29.96 $\pm$ 2.73 ^c	51.99 $\pm$ 2.25 ^c

*Values represent the mean $\pm$ SD of three independent replicates. Different superscript letters (a, b, c) within the same column indicate statistically significant differences between isolates at the same extract concentration, as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

Discussion

Tomato wilt disease, which is caused primarily by the soil-borne pathogen *Fusarium oxysporum* f. sp. *lycopersici*, poses a significant threat to tomato production globally. Traditional control methods for this disease predominantly involve the application of fungicides. While these chemical treatments can effectively reduce disease incidence, they have notable drawbacks, including soil and water pollution, as well as health concerns related to food safety (El-Aswad et al. 2023). Consequently, the development of more sustainable and eco-friendly approaches to combat this devastating disease has become essential (Sivalingam et al. 2024).

To evaluate the effectiveness of *S. officinalis* methanolic extract, four isolates of FOL were isolated and molecularly identified via ITS sequencing. These isolates were designated FOL501, FOL502, FOL503, and FOL504, with the corresponding GenBank accession numbers PQ516909, PQ516910, PQ516911, and PQ516912, respectively. The phylogenetic analysis revealed a close relationship between these isolates and other *Fusarium solani* isolates (e.g., DQ4524541), supporting the morphological identification of the isolates as FOL (Singha et al. 2016).

The EF-1 α -based phylogenetic tree (Fig. 1B) further supported the clustering of FOL501–FOL504 within *F. oxysporum* f. sp. *lycopersici*, with a high bootstrap value of 88. These findings indicate a close evolutionary relationship

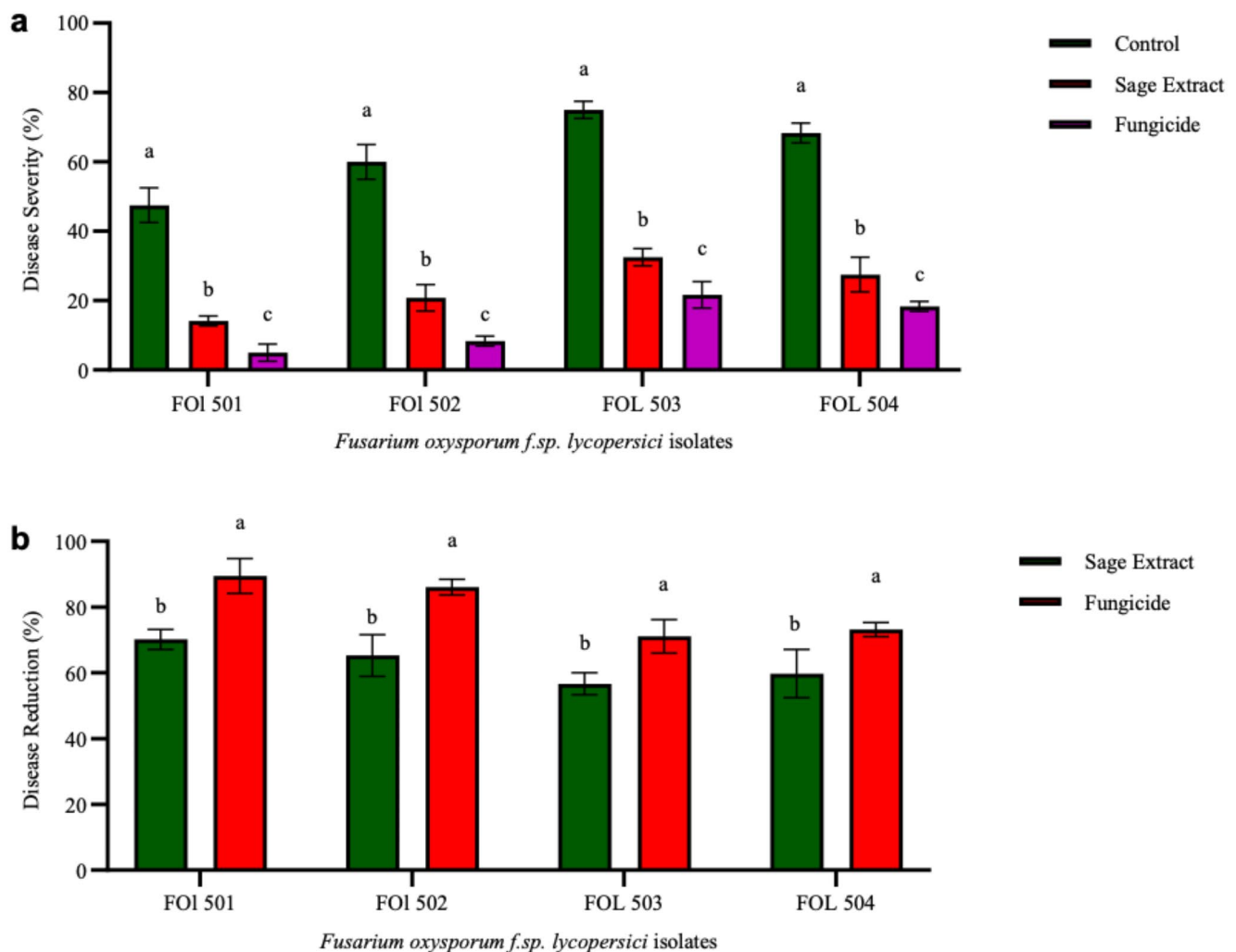


Fig. 4 Disease severity (%) (a) and disease reduction (%) (b) of Fusarium wilt in tomato plants inoculated with four *Fusarium oxysporum* f. sp. *lycopersici* isolates under different treatments

among these tomato-pathogenic isolates and underscore the utility of EF-1 α as a reliable marker for resolving intraspecific relationships within the *Fusarium oxysporum* species complex (Geiser et al. 2004). The consistency between the ITS and EF-1 α trees reinforces the accuracy of the identification and highlights the importance of multilocus phylogenetic analysis for resolving complex taxonomic groups (O'Donnell et al. 2009). In contrast, the TUB2-based phylogenetic tree (Fig. 1C) revealed a slightly lower bootstrap value (58) for the clade containing FOL501–FOL504, suggesting a weaker phylogenetic signal for this marker than for the ITS and EF-1 α . However, the clustering pattern was consistent with that of the other two markers, supporting the classification of these isolates as *F. oxysporum* f. sp. *lycopersici*. The lower bootstrap value may reflect the slower evolutionary rate of the TUB2 gene, which can sometimes result in reduced resolution for closely related isolates (O'Donnell et al. 2009). Despite this, the congruence among

all three markers strengthens the reliability of the phylogenetic analysis and provides a comprehensive understanding of the genetic relationships among the isolates.

Furthermore, the virulence of these isolates was assessed on the basis of the presence or absence of tomato genes (*Tom1*, *Tom2*, *Tom3*, *Tom4*, and *Tom5*) (Pareja-Jaime et al. 2008b). This screening process provides some insights into genetic diversity. The presence or absence of specific tomatinase genes across different isolates influences their pathogenicity and interaction with tomato plants. Tominase enzymes are crucial for the degradation of α -tomatine, a glycoalkaloid produced by tomato plants, as a defense mechanism against pathogens. The ability to metabolize α -tomatine allows certain pathogens to overcome this plant defense, facilitating infection and colonization (Gomaa et al. 2024). The results obtained with the four isolates suggest that while some isolates possess a full complement of tomatinase genes, others may lack specific genes that could

Table 5 Prediction scores of the molecular docking of methanol extract compounds

Code	Compound	Predict score (Kcal/mol)
1 m	Eucalyptol	-3.89
2 m	Camphor	-3.77
3 m	Isoborneol	-3.84
4 m	α -Terpineol	-4.09
5 m	Bornyl acetate	-4.25
6 m	α -Terpinyl acetate	-4.62
7 m	Caryophyllene	-4.08
8 m	Aromadendrene	-4.1
9 m	α -Caryophyllene	-3.93
10 m	Spathulenol	-4.48
11 m	Caryophyllene oxide	-3.97
12 m	10-Undecenoic acid	-4.58
13 m	Viridiflorol	-3.85
14 m	Caryophyllene oxide	-3.42
15 m	Fenretinide	-6.59
16 m	Neophytadiene	-5.52
17 m	Pentadecanoic acid	-5.63
18 m	Palmitic acid	-6.43
19 m	Linolenic acid	-5.38
20 m	Palmitelaidic acid	-5.17
21 m	Phytol	-5.88
22 m	Linoelaidic acid	-6.31
23 m	Oleic acid	-5.59
24 m	Petroselinic acid	-5.31
25 m	Octadecanoic acid	-6.35
26 m	Podocarpa-1,8,11,13-tetraen	-4.68
27 m	12-O-Methylcarnosol	-4.58
28 m	Isocarnosol	-5.1
29 m	9-Anthracenol	-5.03
30 m	9,10-Dimethoxyanthro	-5.14
31 m	Carnosol	-5.02
32 m	Gibberellic acid	-4.39
33 m	α -D-Glucopyranoside	-8.35
34 m	$\psi\psi$ -Carotene, 2-tetrahydro-1-dimethoxy	-7.9
35 m	Oleic acid	-8.69
36 m	12-O-Methylcarnosol	-6.45
37 m	9-Hexadecenoic acid	-7.34

affect their virulence. The presence of multiple tomatinase genes in FOL 504 indicates potentially greater adaptability to tomato defenses than the other isolates do. This variability in pathogenicity among the four obtained isolates made them suitable for further evaluation of the antifungal activity of *S. officinalis* extract.

Phytochemical analysis of the *S. officinalis* methanolic extract revealed important findings regarding its antioxidant properties and phenolic content. Several studies have reported similar results, highlighting the total phenolic content (TPC) and total flavonoid content (TFC) in various extracts of *S. officinalis*. For example, a study indicated that

the TPC of *S. officinalis* ranged from 2.02 to 184 mg GAE/g dry weight, with specific analyses showing values close to 64.15 mg GAE/g, as reported in our study (Moshari-Nasirkandi et al. 2024). Additionally, the total flavonoid content was found to be approximately 54.04 mg QE/g, which aligns with findings that emphasize the significant presence of flavonoids in sage extracts (Khare et al. 2021; Moshari-Nasirkandi et al. 2024). The antioxidant activity of the methanolic extract, reported in our study at 97.04%, is consistent with previous research that demonstrated the strong antioxidant properties of *S. officinalis*, particularly through various assays, such as DPPH radical scavenging (Khare et al. 2021; Lima et al. 2007). Furthermore, the total antioxidant capacity (TAC) of 108.10 mg AAE/g aligns with studies that have quantified the antioxidant capacity of sage extracts via different methodologies (Abdelkader et al. 2015; Khare et al. 2021). Additionally, the obtained GC-mass analysis results are consistent with those of previous studies that have documented the rich chemical composition of *S. officinalis*, emphasizing its antimicrobial activities, such as Gad et al. (2022). Overall, these phytochemical analyses of *S. officinalis* methanolic extract emphasize its high phenolic and flavonoid contents, as well as its robust antioxidant activity, making it a valuable candidate for further antimicrobial.

To evaluate the antifungal potency of the *S. officinalis* methanolic extract against FOL, mycelial growth inhibition (mg/ml) and spore germination (%) were examined. Our data demonstrate a concentration-dependent antifungal effect of *S. officinalis* extract, with significant variability among isolates, as Fol 501 is the most susceptible isolate, showing the greatest mycelium inhibition (43.67–88.67%) and spore germination reduction (34.15–84.51%) across all concentrations (2.5–20 mg/ml). Fol 502 exhibited moderate susceptibility, whereas Fol 503 and Fol 504 presented significantly lower inhibition levels. Additionally, our in vivo data demonstrated that *S. officinalis* extract effectively reduces the severity of tomato wilt diseases caused by various FOL isolates. The application of this extract resulted in a significant decrease in disease severity and an increase in the preventive percentage across the four tested FOL isolates. These findings align with previous research indicating that plant extracts, particularly those from *S. officinalis*, possess antifungal properties capable of mitigating wilt diseases in tomato plants (Abo-Elyousr et al. 2022; Neela et al. 2014).

To gain deeper insight into the mechanism underlying the antifungal activity of *S. officinalis* extract against FOL, we performed an in silico molecular docking analysis (Sharma et al. 2023). Our molecular docking analysis successfully identified several key phytochemicals in *S. officinalis*. These compounds include oleic acid, α -D-glucopyranoside,

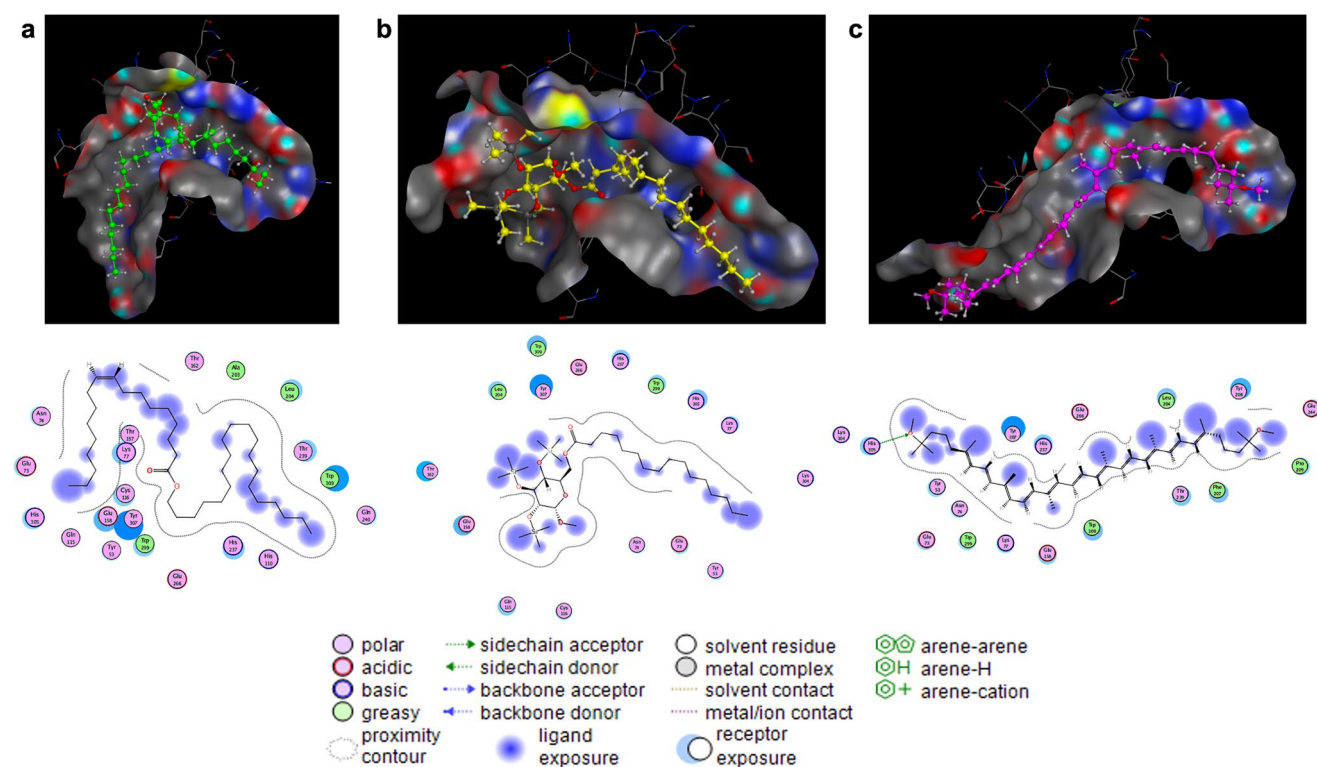


Fig. 5 3D and 2D results of the interaction between (a) oleic acid and the tomatoase endo-1, 4-beta-glycosidase receptor. (b) α -D-Glucopyranoside and the Tomatinase, endo-1, 4-beta-glycosidase receptor. (c)

$\psi\psi$ -Carotene, 2-tetrahydro-1-dimethoxy and the Tomatinase, endo-1, 4-beta-glycosidase receptor

and ψ -carotene (2-tetrahydro-1-dimethoxy). These phytochemicals strongly bind to the enzymes tomatinase and endo-1,4-beta-glycosidase. This is novel and has never been investigated previously, which can explain the antifungal activity of this extract against FOL.

Overall, these findings suggest that these compounds may play a significant role in the therapeutic potential of *S. officinalis*. These findings highlight the potential of *S. officinalis* as an eco-friendly alternative to synthetic fungicides for managing FW in tomato crops. These results suggest that *S. officinalis* methanolic extract could be a valuable eco-friendly solution for managing tomato wilt diseases. This study provides a new sustainable approach to crop protection.

Conclusion

In conclusion, this study demonstrated that the *S. officinalis* methanolic extract exhibited potent antifungal activity against *F. oxysporum*. Making it a promising eco-friendly alternative to chemical fungicides for managing Fusarium wilt in tomato crops. We found that the phytochemical composition of *S. officinalis* extract is rich in phenolic compounds, terpenoids, and fatty acids. This contributes to its

antifungal efficacy, as evidenced by significant mycelial and spore germination inhibition in vitro and reduced disease severity in vivo for four FOL isolates of different virulence levels. Molecular docking further supports the potential mechanism of action, highlighting strong interactions between key phytochemicals detected by GC and fungal virulence factors. These findings underscore the potential of *S. officinalis* extract not only as an effective biocontrol agent but also as a sustainable approach to safeguarding tomato production while mitigating the environmental and ecological drawbacks associated with synthetic fungicides.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s42161-025-01910-5>.

Acknowledgements The authors express their sincere thanks to the Faculty of Agriculture (El-Shatby), Alexandria University, Egypt, for providing the necessary research facilities.

Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB). Open access funding was provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB) for publication. This research received no external funding.

Data availability Data available on request from the authors.

Declarations

Ethical approval and consent for publication Not applicable.

Conflict of interest There are no conflicts to declare.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Aamer HA, Al-Askar AA, Gaber MA, El-Tanbouly R, Abdelkhalek A, Behiry S, Elsharkawy MM, Kowalczewski PL, El-Messeiry S (2023) Extraction, phytochemical characterization, and antifungal activity of salvia Rosmarinus extract. *Open Chem* 21(1):20230124
- Abdelaziz AM, Elrefaey AA, Sharaf MH, Al-Qthanin RN, Attia MS (2024) Enhancing tomato plant immune responses to fusarium wilt disease by red seaweed Jania Sp. *Sci Rep* 14(1):18052
- Abdelkader M, Ahcen B, Rachid D, Hakim H (2015) Phytochemical study and biological activity of Sage (*Salvia officinalis* L.). *Int J Bioeng Life Sci* 8(11):1253–1257
- Abdelkhalek A, Al-Askar AA, Elbeaino T, Moawad H, El-Gendi H (2022) Protective and curative activities of *Paenibacillus polymyxa* against zucchini yellow mosaic virus infestation in squash plants. *Biology* 11(8). <https://doi.org/10.3390/biology11081150>
- Abo-Elyousr KAM, Ali EF, Sallam NMA (2022) Alternative control of tomato wilt using the aqueous extract of *Calotropis procera*. *Horticulturae* 8(3):197
- Bakar MFA, Mohamed M, Rahmat A, Fry J (2009) Phytochemicals and antioxidant activity of different parts of *Bambangan* (*Mangifera pajang*) and *Tarap* (*Artocarpus odoratissimus*). *Food Chem* 113(2):479–483
- Cheng L, Liu JR, Liu JM, Guo D, Deng F, Bian Q, Zhang H, Han X, Ali AS, Zhang WH, Zhang MZ, Gu YC (2023) Design, synthesis, antifungal activity and molecular Docking of ring-opened Pimpinine derivative containing (thio)amide structure. *Pest Manag Sci* 79(6):2220–2229. <https://doi.org/10.1002/ps.7400>
- De Oliveira JR, Vilela P, Almeida RB, de Oliveira A, Carvalho FE, Camargo CAT, Jorge SEA, AOC, de Oliveira LD (2019) Antimicrobial activity of noncytotoxic concentrations of *salvia officinalis* extract against bacterial and fungal species from the oral cavity. *Gen Dent* 67(1):22–26
- Egel DS, Martyn RD (2007) Fusarium wilt of watermelon and other cucurbits. *Plant Health Instr* 07. <https://doi.org/10.1094/PHI-I-2007-0122-01>
- El-Aswad AF, Aly MI, Alsahaty SA, Basyony ABA (2023) Efficacy evaluation of some fumigants against fusarium oxysporum and enhancement of tomato growth as elicitor-induced defense responses. *Sci Rep* 13(1):2479
- Gaber MA, Wagih EE, Shehata MRA, Fahmy MM, Wahab HA (2019) Detection and characterization of botrytis cinerea isolates from vegetable crops in Egypt. *Int J Phytopathol* 8(3):77–85. <https://doi.org/10.33687/phytopath.008.03.2945>
- Gaber MA, El-Bebany AF, Fahmy MM, Aboshosha SS, Wagih EE (2022) Pathological and molecular variability of *F. sarium oxysporum* F. sp *lycopersici* infecting tomato in Egypt. *Egypt Acad J Biol Sci H Bot* 13(2):275–287
- Gad HA, Mamadaliyeva RZ, Khalil N, Zengin G, Najar B, Khojimatov OK, Musayeb A, Ashour NM, ML, Mamadaliyeva NZ (2022) GC-MS chemical profiling, biological investigation of three salvia species growing in Uzbekistan. *Molecules* 27(17):5365
- Geiser DM, Jiménez-Gasco DM, Kang M, Makalowska S, Veeraraghavan I, Ward N, Zhang TJ, Kuldau N, G. A., O'donnell K (2004) FUSARIUM-ID V. 1.0: A DNA sequence database for identifying fusarium. *Eur J Plant Pathol* 110:473–479. <http://fusarium.cbio.psu.edu>
- Ghorbani A, Esmailizadeh M (2017) Pharmacological properties of *salvia officinalis* and its components. *J Traditional Complement Med* 7(4):433–440
- Gomaa FH, El-Bebany AF, Shahda W, Komeil D (2024) Molecular and F.nctional characterization of Tomatinase secreted by isolates of *F.sarium oxysporum* F. Sp. *lycopersici*, the causal of vascular wilt of tomato. *Egypt J Phytopathol* 52(2):25–34
- Gordon TR (2017) Fusarium oxysporum and the fusarium wilt syndrome. *Annu Rev Phytopathol* 55(1):23–39
- Green MR, Sambrook J (2012) Molecular cloning: a laboratory manual, 4th edn. Cold Spring Harbor Laboratory Press
- Grover RK, Moore JD (1962) Toximetric studies of fungicides against the brown rot organisms. *Sclerotinia fructicola* and *S. laxa*
- Himabindu P, Kumar V (2021) Methods for management of fusarium wilt in tomato. *Int J Curr Microbiol App Sci* 10(01):363–371
- Jazo Z, Glumac M, Pastar V, Bektic S, Radan M, Carev I (2023) Chemical Composition and Biological Activity of *Salvia officinalis* L. Essential Oil. *PLANTS-BASEL* 12(9)
- Khare R, Upmanyu N, Jha M (2021) Exploring the potential effect of methanolic extract of *salvia officinalis* against UV exposed skin aging: in vivo and in vitro model. *Curr Aging Sci* 14(1):46–55
- Khedher MR, Ben, Khedher S, Ben, Chaieb I, Tounsi S, Hammami M (2017) Chemical composition and biological activities of *salvia officinalis* essential oil from Tunisia. *EXCLI J* 16:160
- Krif G, El Aissami A, Zoubi B, Dababat AA, Khfif K, Lahlali R, Mokriani F (2024) Evaluation of the nematocidal effect of some essential oils on *meloidogyne javanica* under in vitro and in vivo conditions. *Crop Health J*, 76(6):1519–1528.
- Kumar M, Kajal, Taranum N, Khyati, Balan B, Kumar P, Dhaulaniya AS (2024) Antifungal potential of bioactive compounds derived from microbes and other natural sources: challenges and future scope. *Advances in antifungal drug development. Natural Products with Antifungal Potential*, pp 591–609
- Kursa W, Jamiołkowska A, Wyrostek J, Kowalski R (2022) Antifungal effect of plant extracts on the growth of the cereal pathogen *fusarium* spp.—an in vitro study. *Agronomy* 12(12):3204
- Lahlou Y, Moujabbir S, Aboukhalaf A, El Amraoui B, Bamhaoud T (2023) Antibacterial activity of essential oils of *salvia officinalis* growing in Morocco. *Roczniki Państwowego Zakładu Higieny* 74(4)
- Lima CF, Valentao PCR, Andrade PB, Seabra RM, Fernandes-Ferreira M, Pereira-Wilson C (2007) Water and methanolic extracts of *salvia officinalis* protect HepG2 cells from t-BHP induced oxidative damage. *Chemico-Biol Interact* 167(2):107–115
- López-Zapata SP, García-Jaramillo DJ, López WR, Ceballos-Aguirre N (2021) Tomato (*Solanum lycopersicum* L.) and *F.sarium oxysporum* F. Sp. *lycopersici* interaction. A review. *Revista UDCA Actualidad Divulgación Científica* 24(1)

- Moshari-Nasirkandi A, Iaccarino N, Romano F, Graziani G, Alirezalu A, Alipour H, Amato J (2024) Chemometrics-based analysis of the phytochemical profile and antioxidant activity of salvia species from Iran. *Sci Rep* 14(1):17317
- Neela FA, Sonia IA, Shamsi S (2014) Antifungal activity of selected medicinal plant extract on fusarium oxysporum schlechtthe causal agent of fusarium wilt disease in tomato. *American Journal of Plant Sciences*
- O'donnell K, Corby Kistler H, Cigelnik, E, & Ploetz, R. C. (1998). Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. In *applied biological sciences communicated by R.* (vol. 95). <https://doi.org/10.1073/pnas.95.5.2044>
- O'donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus fusarium are nonorthologous
- O'Donnell K, Gueidan C, Sink S, Johnston PR, Crous PW, Glenn A, Riley R, Zitomer NC, Colyer P, Waalwijk C, van der Lee T, Moretti A, Kang S, Kim HS, Geiser DM, Juba JH, Baayen RP, Cromey MG, Bithell S, Sarver BAJ (2009) A two-locus DNA sequence database for typing plant and human pathogens within the fusarium oxysporum species complex. *Fungal Genet Biol* 46(12):936–948. <https://doi.org/10.1016/j.fgb.2009.08.006>
- Pareja-Jaime Y, Roncero MIG, Ruiz-Roldán MC (2008a) Tomatinase F.om F.sarium oxysporum F. Sp. lycopersici is required F.r F.11 virulence on tomato plants. *Mol Plant Microbe Interact* 21(6):728–736. <https://doi.org/10.1094/MPMI-21-6-0728>
- Pareja-Jaime Y, Roncero MIG, Ruiz-Roldán MC (2008b) Tomatinase F.om F.sarium oxysporum F. Sp. lycopersici is required F.r F.11 virulence on tomato plants. *Mol Plant Microbe Interact* 21(6):728–736
- Porte A, Godoy RLO, Maia-Porte LH (2013) Chemical composition of Sage (*Salvia officinalis* L.) essential oil from the Rio de Janeiro state (Brazil). *Revista Brasileira De Plantas Medicinai* 15:438–441
- Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem* 269(2):337–341
- Rongai D, Milano F, Sciò E (2012) Inhibitory effect of plant extracts on conidial germination of the phytopathogenic fungus fusarium oxysporum
- Shakam HM, Gaber MA (2024) Biodegradation of petroleum hydrocarbon and related molecular microbiology analysis of some fungal species. *Alexandria J Agricultural Sci* 69(3):316–327
- Sharma AD, Kaur I, Chauhan A (2023) Molecular Docking studies of principal components and in vitro inhibitory activities of Rosmarinus officinalis essential oil against Aspergillus flavus, Aspergillus fumigatus and mucor indicus. *Phytomedicine Plus* 3(4):100493
- Singh P, Sudhanshu PK, Revathi KG, Maheswari M, Bonde SD, Bharanidharan R (2023) Molecular Docking studies of different phytochemicals obtained from medicinal plants of Uttarakhand region for identification of potential inhibitors against mucormycosis causing fungal species. *J Appl Nat Sci* 15(1):162–177
- Singha IM, Kakoty Y, Unni BG, Das J, Kalita MC (2016) Identification and characterization of fusarium Sp. using ITS and RAPD causing fusarium wilt of tomato isolated from Assam, North East India. *J Genetic Eng Biotechnol* 14(1):99–105
- Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Viticult* 16(3):144–158
- Sivalingam S, Golla G, Arunachalam L, Singh T, Karthikeyan G, Shanthi A, Malaichamy K (2024) Encapsulation of essential oil to prepare environment F.iendly nanobio-fungicide against F.sarium oxysporum F. Sp. lycopersici: an experimental and molecular dynamics approach. *Colloids Surf A* 681:132681
- Song W, Zhou L, Yang C, Cao X, Zhang L, Liu X (2004) Tomato fusarium wilt and its chemical control strategies in a hydroponic system. *Crop Prot* 23(3):243–247
- Srinivas C, Nirmala Devi D, Narasimha Murthy K, Mohan CD, Lakshmeesha TR, Singh BP, Kalagatur NK, Niranjana SR, Hashem A, Alqarawi AA, Tabassum B, Abd_Allah EF, Nayaka C, S (2019) Fusarium oxysporum F. Sp. lycopersici causal agent of vascular wilt disease of tomato: biology to diversity– A review. *Saudi J Biol Sci* 26(7):1315–1324. <https://doi.org/10.1016/j.sjbs.2019.06.002>
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: Guide Methods Appl* 18(1):315–322
- Woisky RG, Salatino A (1998) Analysis of propolis: some parameters and procedures for chemical quality control. *J Apic Res* 37(2):99–105
- Youssef NH, Qari SH, Matar S, Hamad NA, Dessoky ES, Elshaer MM, Sobhy S, Abdelkhalek A, Zakaria HM, Heflish AA, Elsamra IA, Behiry SI (2021) Licorice, Doum, and banana Peel extracts inhibit Aspergillus flavus growth and suppress metabolic pathway of aflatoxin b1 production. *Agronomy* 11(8):1–16. <https://doi.org/10.3390/agronomy11081587>
- Zaccardelli M, Pane C, Caputo M, Durazzo A, Lucarini M, Silva AM, Severino P, Souto EB, Santini A, De Feo V (2020) Sage species case study on a spontaneous mediterranean plant to control phytopathogenic fungi and bacteria. *Forests* 11(6):704

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.