

Dual Efficacy of *Aspergillus oryzae* SAF1 in Suppressing Fusarium Wilt and Anthracnose and Promoting Tomato and Chili Growth

(Keberkesanan Berganda *Aspergillus oryzae* SAF1 dalam Menekan Layu Fusarium dan Antraknosa serta Menggalakkan Pertumbuhan Tomato dan Cili)

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ABSTRACT

The development of sustainable alternatives to chemical fungicides is imperative for managing economically significant plant diseases such as Fusarium wilt in tomato and anthracnose in chili. This study aimed to isolate and screen native fungi for antagonistic activity and biocontrol traits, identify the most promising candidate, evaluate its plant growth-promoting capabilities, and assess its efficacy in suppressing disease and enhancing growth in pot experiments under open greenhouse conditions. *In vitro* screening showed that SAF1 exhibited antagonistic activity against *Fusarium oxysporum* f. sp. *lycopersici*, *Colletotrichum acutatum*, *C. capsici*, and *C. gloeosporioides*, alongside biocontrol traits including siderophores and hydrolytic enzymes (chitinase, cellulase, amylase, and lipase) production. Morphological and ITS analyses identified the isolate as *Aspergillus oryzae* SAF1. Based on this species-level classification, *A. oryzae* SAF1 presents a potentially safe profile for agriculture. *A. oryzae* SAF1 exhibited plant growth-promoting traits, including the synthesis of indole-3-acetic acid (IAA) and ammonia, complemented by its ability to grow and form a clear zone on nitrogen-free medium, suggesting potential nitrogen fixation, a trait previously unreported for *A. oryzae*. In pot experiments, *A. oryzae* SAF1 application significantly reduced the disease index of Fusarium wilt (to 15.00%) and anthracnose (to 7.50%), while enhancing shoot length and biomass in both crops. This study provided the first evidence of *A. oryzae* as a dual-action agent that simultaneously suppresses anthracnose and promotes growth in chili under pot conditions. These findings demonstrate that *A. oryzae* SAF1 is a highly promising candidate for development as a bioinoculant to sustainably enhance crop protection and growth.

Keywords: Antagonistic fungi; biological control; disease suppression; mycoparasitism; plant growth-promoting fungi

ABSTRAK

Pembangunan alternatif mampan kepada racun kulat kimia adalah penting bagi menguruskan penyakit tumbuhan yang signifikan dari segi ekonomi, seperti layu Fusarium pada tomato dan antraknos pada cili. Kajian ini bertujuan untuk memencil dan menyaring kulat tempatan bagi mengesan aktiviti antagonistik dan ciri kawalan biologi, mengenal pasti calon yang paling berpotensi, menilai keupayaannya menggalakkan pertumbuhan tumbuhan serta menguji keberkesanannya dalam merencat penyakit dan meningkatkan pertumbuhan melalui uji kaji pasu dalam keadaan rumah hijau terbuka. Saringan *in vitro* menunjukkan bahawa SAF1 menunjukkan aktiviti antagonistik terhadap *Fusarium oxysporum* f. sp. *lycopersici*, *Colletotrichum acutatum*, *C. capsici* dan *C. gloeosporioides* serta ciri kawalan biologi termasuk penghasilan siderofor dan enzim hidrolitik (kitinase, selulase, amilase dan lipase). Analisis morfologi dan ITS mengenal pasti pencilan tersebut sebagai *Aspergillus oryzae* SAF1. Berdasarkan pengelasan pada peringkat spesies ini, *A. oryzae* SAF1 menunjukkan profil yang berpotensi selamat untuk kegunaan pertanian. *A. oryzae* SAF1 menunjukkan ciri penggalak pertumbuhan tumbuhan, termasuk sintesis asid indol-3-asetik (IAA) dan ammonia serta keupayaan untuk tumbuh dan membentuk zon jernih pada medium bebas nitrogen yang mencadangkan potensi penetapan nitrogen, satu ciri yang sebelum ini tidak pernah dilaporkan bagi *A. oryzae*. Dalam uji kaji pasu, penggunaan *A. oryzae* SAF1 mengurangkan indeks penyakit layu Fusarium (kepada 15.00%) dan antraknos (kepada 7.50%) secara signifikan, di samping meningkatkan panjang pucuk dan biojisim bagi kedua-dua tanaman tersebut. Kajian ini memberikan bukti pertama bahawa *A. oryzae* bertindak sebagai agen dwi-fungsi yang secara serentak merencat antraknos dan menggalakkan pertumbuhan cili dalam

keadaan pasu. Keputusan ini menunjukkan bahawa *A. oryzae* SAF1 merupakan calon yang sangat berpotensi untuk dibangunkan sebagai bioinokulan bagi meningkatkan perlindungan dan pertumbuhan tanaman secara mampan.

Kata kunci: Kawalan biologi; kulat antagonistik; kulat penggalak pertumbuhan tumbuhan; mikoparasitisme; perencanan penyakit

INTRODUCTION

Fungal infections are responsible for the loss of over 70% of agricultural produce (Deresa & Diriba 2023). Fusarium wilt disease, caused by the fungus *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), is a major vascular disease that poses a significant constraint to *Solanum lycopersicum* (tomato) cultivation. Anthracnose disease, caused by species of the genus *Colletotrichum*, represents a key phytopathological issue in *Capsicum* spp. (chili) production. These fungal phytopathogens can lead to substantial reductions in both the quality and quantity of agricultural yield, and in severe infections, may result in postharvest losses, rendering the produce unmarketable (Adhikari et al. 2020; Yadav, Dubey & Upadhyay 2021). In general, farmers commonly use chemical pesticides to control plant diseases in order to increase crop yields. However, the use of chemical fungicides to prevent fungal pathogens has caused significant problems and poses serious risks to both human health and the environment, especially when chemical residues remain on agricultural products (Deresa & Diriba 2023). Subsequently, researchers discovered a method to control plant pathogenic microorganisms by utilizing antagonistic microbes. Fungal antagonists have gained increasing attention and are being used as alternatives to chemical agents in plant disease management in agriculture.

Fungal antagonists serve as biological control agents that help suppress plant pathogenic microorganisms and promote plant growth. These fungi can survive in the environment, even in the absence of a host plant, and reproduce both sexually and asexually, allowing them to survive and grow rapidly. They exhibit target-specific action (Thambugala et al. 2020), making them suitable for controlling plant pathogens. For example, fungi such as *Aspergillus* and *Rhizopus* have been used to control the fungus *Fol* (Attia et al. 2022), and fungi in the genera *Aureobasidium*, *Epicoccum*, *Trichoderma*, *Curvularia*, and *Clonostachys* have been employed to control *Colletotrichum* species (Shi et al. 2021). Fungal antagonists can control pathogenic fungi through mechanisms such as secreting cell wall-degrading enzymes, producing antimicrobial substances (antifungal metabolites), competing with pathogens for nutrients and space, and inducing resistance. They can also promote plant growth by producing plant hormones, enhancing mineral and nutrient uptake, and inducing systemic resistance (Adeleke et al. 2022; Sun, Shahrajabian & Guan 2025). However, under Thailand's hot and humid climate, previously reported foreign fungal

antagonist strains may not provide full effectiveness in controlling plant diseases. Therefore, it is necessary to study and select local fungal antagonist strains from Thailand for use in disease management, especially for tomato wilt and chili anthracnose, which significantly affect crop cultivation and product quality in the country. The use of these local fungal antagonists may be an important approach toward sustainably reducing the use of chemical pesticides in disease control. Moreover, by selecting strains with beneficial traits, both the quality and quantity of crop yields can be effectively improved.

Therefore, this study aimed to isolate and select potential native antagonistic fungal strains from soil in the Phu Phan National Park, Sakon Nakhon Province, Thailand. The isolated strains were initially screened for their antagonistic activity and biocontrol traits against *Fol* and *Colletotrichum* spp. Subsequently, the most prominent isolate was identified and further characterized for its plant growth-promoting capabilities. Finally, the overall efficacy of this selected isolate in controlling disease and promoting the growth of tomato and chili was evaluated under pot experimental conditions.

MATERIALS AND METHODS

FUNGAL ISOLATION AND GROWTH CONDITION

Fungal isolation was conducted from soil in the Phu Phan National Park, Sakon Nakhon, Thailand, in September 2023. Prior to soil collection, surface plant litter and debris were removed, and the sample was collected at a depth of 0-10 cm. Isolation was performed on Potato Dextrose Agar (PDA; Difco™, USA) supplemented with 100 mg/L ampicillin (Wang et al. 2023), and cultures were incubated at 28 ± 2 °C until growth appeared. Distinct colonies were then subcultured on PDA for 7 days and purified through successive transfers. Anthracnose pathogens (*Colletotrichum acutatum*, *Colletotrichum capsici*, and *Colletotrichum gloeosporioides*) were obtained from the Department of Agriculture, Bangkok, while *Fol* was isolated from infected tomato tissues in Tao Ngoi District, Sakon Nakhon. All isolates were maintained on Potato Dextrose Agar (PDA; Difco™) slants at 4 °C, subcultured on fresh PDA plates, and incubated at 28 °C for 7 days. Spore suspensions of *Colletotrichum* spp. were prepared by adding 10 mL of sterile distilled water to each culture, scraping gently with a sterile glass rod, filtering through sterile Whatman No. 1 paper, and adjusting the concentration to 1×10^7 spores/mL using a hemocytometer. For *Fol*, seven 0.6 cm mycelial

plugs were incubated in 100 mL of sterile distilled water at 28 °C for 5 days at 120 rpm, and the spore concentration was adjusted as mentioned after filtering the suspension through sterile Whatman No. 1 paper to remove mycelial fragments.

SCREENING FOR ANTAGONISTIC ACTIVITY AND BIOCONTROL TRAITS

The antagonistic efficacy was evaluated using the dual culture technique. Mycelial plugs (0.6 cm diameter) were cut from the edge of actively growing colonies of both the pathogen and each fungal isolate. On a fresh 9-cm PDA plate, a pathogen plug was placed 2.0 cm from one edge, and a plug of the tested isolate was placed directly opposite, also 2.0 cm from the edge. Agar plates inoculated with only the pathogen plug served as the negative control. All plates were incubated at 28 °C for 7 days, with four replicates. Colony radial growth of the pathogen was measured along the axis connecting the two plugs in the treatment plates, and along the corresponding radius in the negative control plates. Percent inhibition of radial growth (PIRG) was calculated by the formula (Holkar et al. 2023). $\%PIRG = [(M1-M2)/M1] \times 100$, where M1 = radial growth of the pathogen colony in the negative control, and M2 = radial growth of the pathogen colony in the presence of the tested isolate.

The fungal isolates were evaluated for their potential to produce extracellular hydrolytic enzymes and siderophores. Chitinase activity was assessed by cultivating the isolate on Basal Medium (BM) composed of 0.2% (w/v) dipotassium hydrogen phosphate, 0.2% (w/v) potassium dihydrogen phosphate, 0.05% (w/v) magnesium sulfate heptahydrate, 0.4% (w/v) ammonium sulfate, and 2% (w/v) agar, supplemented with 1% (w/v) colloidal chitin, with the pH adjusted to 5.6 prior to inoculation. A fungal plug was aseptically inoculated at the center of the agar plate and incubated at 28 °C for 8 days. The appearance of a clear zone surrounding the fungal colony indicated the degradation of colloidal chitin due to extracellular chitinase activity (Chung et al. 2022). Cellulase activity was evaluated using PDA plates supplemented with 1% (w/v) carboxymethyl cellulose sodium (CMC-Na), inoculated with a fungal plug and incubated at 28 °C for 3 days. After incubation, the plates were stained with 1% (w/v) Congo red and destained with a 1 N sodium chloride solution; a clear yellowish zone indicated cellulose degradation by extracellular cellulase (Naziz, Das & Sen 2024). For lipase screening, PDA plates containing 1% (v/v) tributyrin were used, and the presence of a clear zone around the colony after 3 days of incubation at 28 °C confirmed extracellular lipase production (Naziz, Das & Sen 2024). Amylase production was tested on PDA plates supplemented with 0.2% (w/v) soluble starch, incubated at 28 °C for 3 days,

and subsequently flooded with iodine solution. A clear zone surrounding the fungal colony, against a blue-black background of unhydrolyzed starch, indicated starch hydrolysis by extracellular amylase (Imran et al. 2021). In addition to hydrolytic enzyme production, siderophore activity was evaluated by inoculating a fungal plug onto a Chrome Azurol S (CAS) agar plate and incubating at 28 °C for 3 days. The formation of an orange halo around the colony indicated siderophore production (Cui et al. 2025).

MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF THE SELECTED ISOLATE

To examine its macroscopic characteristics, the selected isolate was cultured on several diagnostic media, including PDA, Czapek Yeast Autolysate Agar (CYA), and Yeast Extract Sucrose Agar (YES) (Suleiman 2023). The plates were incubated at 28 °C for 7 days. Colony characteristics such as color and texture were recorded. For molecular characterization, the selected isolate was cultured in Potato Dextrose Broth (PDB; Difco™) at 28 °C with shaking at 250 rpm for 3 days. The mycelium was collected and ground using a mortar and pestle with liquid nitrogen added during the grinding process. Genomic DNA was extracted using a commercial DNA extraction kit (Thermo Scientific) and then quantified. PCR amplification of the ITS region was performed, and the resulting amplicons were verified by gel electrophoresis before being sent for sequencing at Macrogen Inc. (South Korea). The obtained ITS sequences were analyzed using BioEdit® 7.2 and FinchTV software, then compared to the NCBI database using the BLAST tool to identify the fungal species.

EVALUATION OF PLANT GROWTH-PROMOTING TRAITS OF THE SELECTED ISOLATE

The plant growth-promoting potential of the selected isolate was further evaluated through assays for indole-3-acetic acid (IAA) production, ammonia production, and nitrogen fixation. To prepare the inoculum, a 7-day-old culture of the isolate was washed with sterile distilled water, and the conidia and mycelia were gently loosened with a sterile glass rod. The mixture was filtered through Whatman No. 1 filter paper to remove mycelial fragments, and the final concentration was standardized to 1×10^7 spores/mL using a hemocytometer. For IAA production, a 1 mL aliquot of this standardized spore suspension was inoculated into 100 mL of PDB supplemented with 100 mg of L-tryptophan in a 250 mL Erlenmeyer flask and incubated at 28 °C with shaking at 150 rpm for 7 days. After 7 days of incubation, a 3 mL culture sample was collected to determine IAA concentration. After centrifugation at $5,635 \times g$ for 10 min, 1 mL of the resulting supernatant was mixed with 2 mL of Salkowski's reagent and

incubated in the dark at 28 °C for 30 min. IAA concentration was then determined spectrophotometrically at 530 nm using a standard curve of known IAA concentrations (Arora et al. 2024). For ammonia production, a 1 mL aliquot of standardized spore suspension was inoculated into 100 mL of 4% (w/v) peptone water and incubated at 28 °C with shaking at 150 rpm for 12 days. Subsequently, 0.5 mL of Nessler's reagent was added to 2.5 mL of the culture supernatant. The ammonia concentration was measured spectrophotometrically at 420 nm, using a standard curve prepared with known concentrations of ammonium sulfate (Jassim et al. 2023). Nitrogen fixation ability was tested by inoculating a fungal plug onto Ashby solid medium (pH 7.0) consisting of 2.0% (w/v) sucrose, 0.02% (w/v) potassium dihydrogen phosphate, 0.02% (w/v) sodium chloride, 0.02% (w/v) magnesium sulfate heptahydrate, 0.01% (w/v) potassium sulfate, 0.5% (w/v) calcium carbonate, and 1.5% (w/v) agar, followed by incubation at 28 °C for 7 days. The formation of a clear zone around the fungal colony indicated nitrogen-fixing capability (Cui et al. 2025).

EVALUATION OF THE SELECTED ISOLATE FOR DISEASE CONTROL AND PLANT GROWTH PROMOTION IN POT EXPERIMENTS

The efficacy of the selected isolate was evaluated in two host-pathogen systems through pot experiments conducted in an open greenhouse at Sakon Nakhon Rajabhat University, Thailand. Over the two-month experimental period, the average temperature and relative humidity were 22.6 °C and 63.25%, respectively. The first system assessed the control of *Fol* and growth promotion in tomato. The second system evaluated the control of *C. gloeosporioides* and growth promotion in chili. Among the three *Colletotrichum* spp. tested *in vitro*, *C. gloeosporioides* was selected for this experiment as it is a primary anthracnose pathogen in chili in Thailand and has limited available biocontrol information.

Twenty-one-day-old seedlings were transplanted into individual pots. The potting medium for each pot was prepared by mixing 1.2 kg of sterile peat moss (autoclaved at 121 °C for 20 min) with 1.2 g of 15:15:15 fertilizer, achieving 0.18 g N/pot, 0.08 g P/pot, and 0.15 g K/pot. The experiment was conducted using a completely randomized design (CRD) with ten replications per treatment and one plant per pot. All pots were watered daily with 250 mL of water. The four experimental treatments were as follows: (T1) plants receiving neither the selected isolate nor pathogens (healthy control); (T2) plants treated only with the selected isolate; (T3) plants inoculated only with fungal pathogens (infected control); and (T4) plants treated with both the selected isolate and the fungal pathogens.

Both the selected isolate and the fungal pathogens were applied as a 50 mL spore suspension (1×10^7

spores/mL) by applying the suspension to the soil around the plant roots. At any given application time, plants in treatments not designated to receive a fungal inoculum were instead mock-inoculated with an equivalent volume (50 mL) of sterile distilled water to ensure consistent handling. The application schedule for the selected isolate (T2 and T4) occurred on the day of transplanting (day 0), and again at 10, 20, and 30 days post-transplanting; correspondingly, T1 and T3 received sterile distilled water on these days. Pathogen inoculation for T3 and T4 was performed once at 10 days post-transplanting, while T1 and T2 received a corresponding mock inoculation with sterile distilled water on that day. At 60 days after transplanting, disease index (DI) for Fusarium wilt and anthracnose was calculated based on a 0-5 rating scale, following Attia et al. (2022) and Wu et al. (2024), respectively. Growth parameters, including shoot length (cm), fresh weight (g), and dry weight (g), were also measured.

STATISTICAL ANALYSIS

Prior to analysis, data obtained from the pot experiments, including DI, shoot length, and plant biomass (fresh and dry weights) for both tomato and chili, were subjected to normality and homogeneity of variances tests using the Shapiro-Wilk and Levene's tests, respectively. For parameters that met both assumptions, data were analyzed using one-way ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$). In cases where the normality assumption was satisfied but homogeneity of variances was violated, Welch's ANOVA combined with the Games-Howell post-hoc test was applied. For data that did not meet the normality assumption, the non-parametric Kruskal-Wallis test was utilized, and pairwise comparisons were performed using the Mann-Whitney U test. Regardless of the statistical test applied, all descriptive data are presented as means $\pm$ standard deviations to maintain consistency and facilitate biological interpretation. All statistical analyses were performed using SPSS version 29.

RESULTS AND DISCUSSION

ISOLATION AND SCREENING FOR ANTAGONISTIC ACTIVITY AND BIOCONTROL TRAITS

Among all recovered isolates, a prominent fungal strain, designated as SAF1, was selected for further investigation due to its broad-spectrum inhibition against *Fol* and three *Colletotrichum* spp. and its successful expression of all evaluated biocontrol traits. The %PIRG against *Fol*, *C. acutatum*, *C. capsici*, and *C. gloeosporioides* was $43.21 \pm 0.48\%$, $43.12 \pm 0.54\%$, $40.28 \pm 0.77\%$, and $42.03 \pm 0.55\%$, respectively (Figure 1(A)-1(D)). Furthermore, the enzyme assay showed that SAF1 exhibited positive activity for chitinase, cellulase, amylase, and lipase, as indicated

by the formation of clear hydrolysis zones around the colonies on their respective selective media (Figure 2(A)-2(D)). The diameters of these hydrolysis zones were measured at 18.62 ± 0.68 mm for chitinase, 63.25 ± 0.79 mm for cellulase, 34.46 ± 0.38 mm for amylase, and 23.63 ± 0.39 mm for lipase. Additionally, siderophore production was evaluated on CAS agar plates, where a distinct orange halo measuring 43.10 ± 1.32 mm in diameter was observed around the fungal colony (Figure 2(E)).

These results align with previous reports highlighting the potential of fungal species, such as *Aspergillus*, *Rhizopus*, and *Trichoderma*, as biological control agents against *Fol* (Attia et al. 2022; Choi & Ahsan 2022; Khuong et al. 2023; Sanneboyina & Audipudi 2024) and *Colletotrichum* spp. (Boukaew et al. 2024; Holkar et al. 2023; Peters et al. 2020; Vu et al. 2021). The observed broad-spectrum inhibition by SAF1 is likely driven by its robust biocontrol properties. Specifically, the secretion of extracellular hydrolytic enzymes, including chitinase, cellulase, lipase, and amylase, enables fungal antagonists to directly degrade the cell walls, spores, and hyphae of phytopathogens (Guzmán-Guzmán et al. 2023; Komeil & Saad 2021; Qu et al. 2021; Sun et al. 2024; Wang et al. 2024). Siderophores are organic compounds that can efficiently chelate iron in the form of Fe^{3+} . Iron (Fe^{3+}) is an essential element for all microbial growth and plays a critical role in various biological processes. Fungi capable of producing siderophores have a higher ability to compete for iron from the environment than those that cannot synthesize these compounds. When siderophores bind to Fe^{3+} , the complex is transported back into the microbial

cell that produced it, allowing the microorganism to acquire the iron necessary for survival. Pathogenic microbes that cannot utilize these siderophores or are less efficient in iron acquisition become iron-deficient, which inhibits their growth and pathogenicity. Siderophore production is thus a natural mechanism that effectively controls pathogenic microorganisms (Attia et al. 2023; Bhardwaj et al. 2023; Pecoraro et al. 2022). Taken together, the ability of SAF1 to produce a range of hydrolytic enzymes and siderophores indicates its strong potential as a promising biocontrol agent.

IDENTIFICATION OF SAF1

Macroscopic characteristics of SAF1 were observed on different culture media, including PDA, CYA, and YES. These three culture media are standard media commonly used for studying colony morphology and sporulation of fungi. The results showed that the isolate SAF1 exhibited distinct macroscopic colony characteristics and sporulation patterns, depending on the type of culture medium used after 7 days of incubation. Observations showed that SAF1 produced dark brown to black pigmentation on PDA medium (Figure 3(A)), whereas mustard-colored conidia were observed on CYA (Figure 3(B)) and YES media (Figure 3(C)). These characteristics are consistent with published descriptions for *Aspergillus oryzae* (Awad et al. 2023; Suleiman 2023), which was subsequently confirmed by molecular identification. Molecular identification based on the ITS gene confirmed that the fungal isolate SAF1 has 100% similarity to *A. oryzae* NRRL 447 (GenBank Accession No. NR_135395). Therefore, the isolate was identified

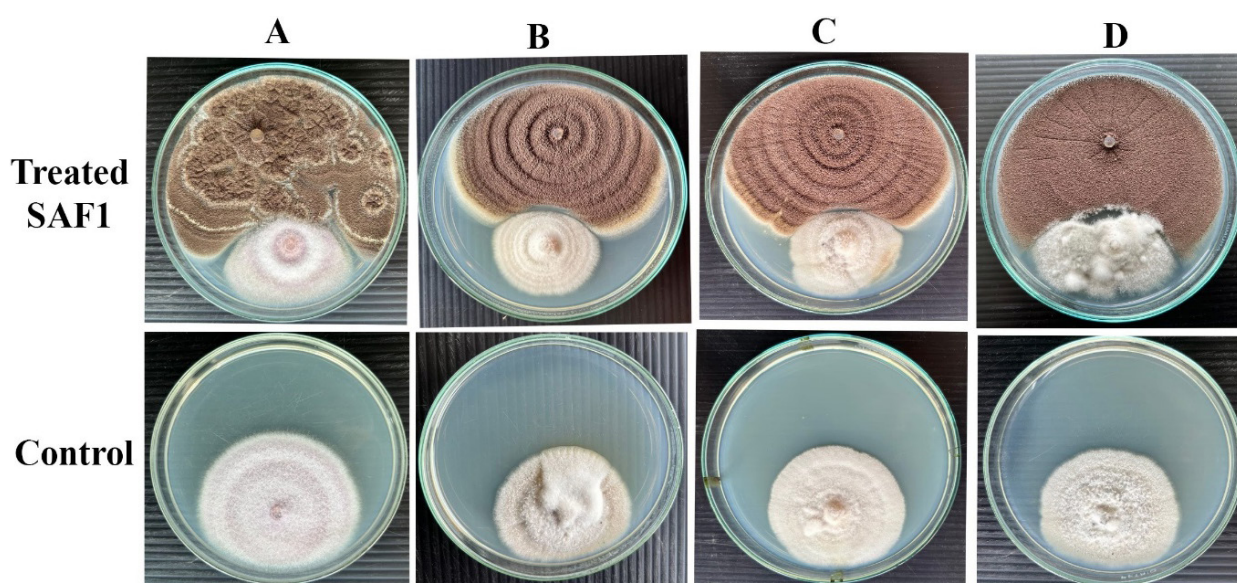


FIGURE 1. Antagonistic activity of SAF1 against *Fol* (A), *C. acutatum* (B), *C. capsici* (C), and *C. gloeosporioides* (D) using the dual culture technique

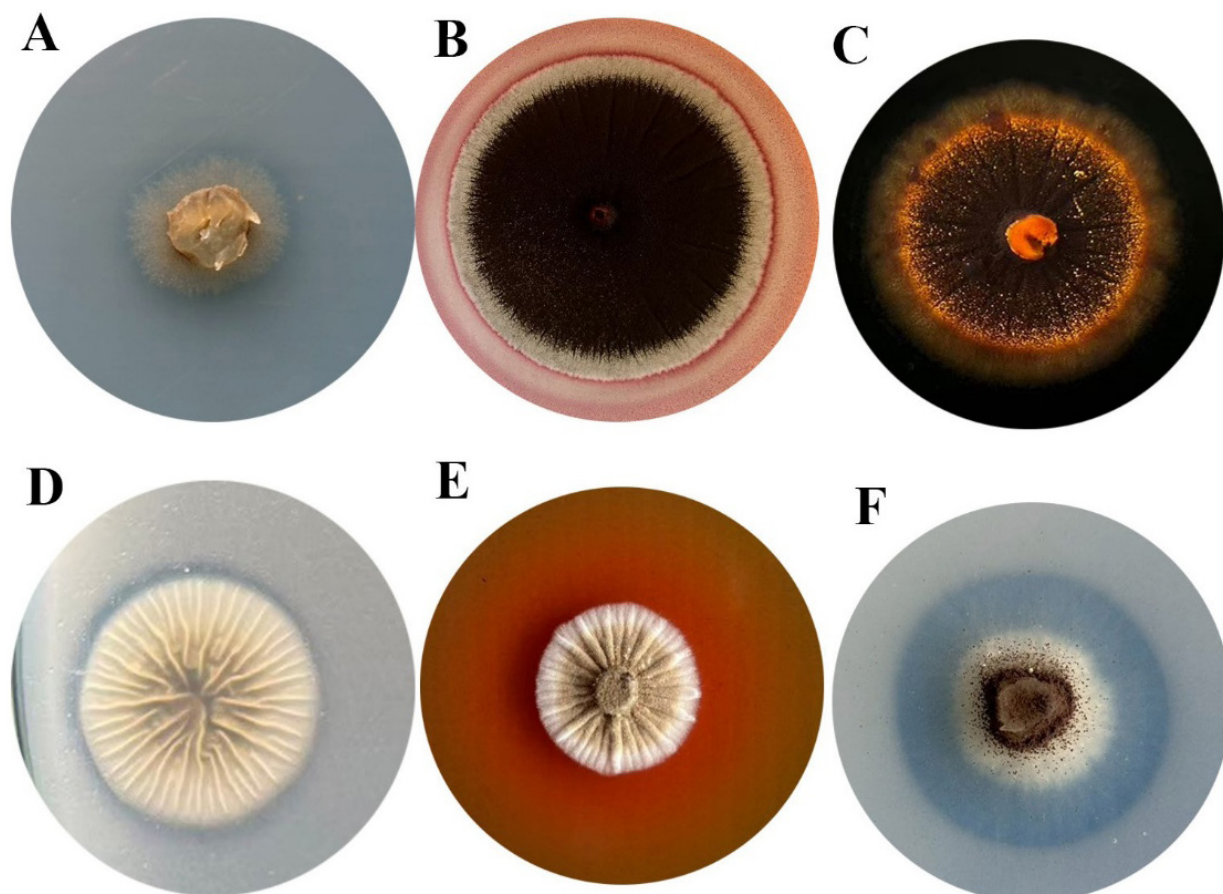


FIGURE 2. Multifunctional traits of SAF1: enzymatic activities of chitinase (A), cellulase (B), amylase (C), and lipase (D); siderophore production (E); and nitrogen fixation (F)

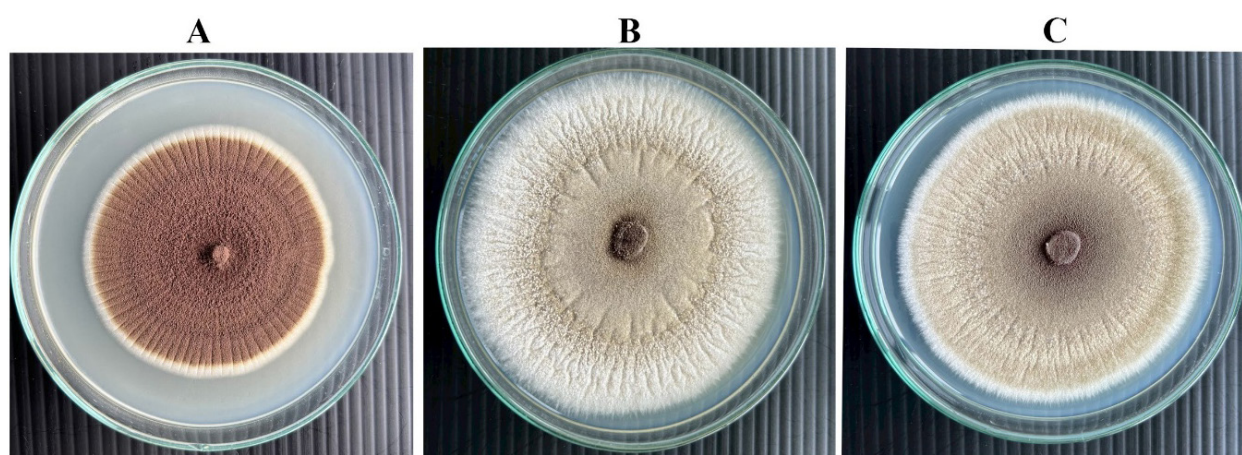


FIGURE 3. Colony and conidial pigmentation of SAF1 on PDA (A), CYA (B), and YES (C)

as *A. oryzae* SAF1 (GenBank Accession No. PX381338). Notably, strain NRRL 447 serves as the official neotype for this species and has a well-documented history of being isolated from koji, a traditional fermenting agent safely utilized in food production for centuries (Wicklow, McAlpin & Peterson 2002). Generally, *A. oryzae* is widely recognized as an atoxigenic species with a negligible risk of producing harmful toxins (Wang et al. 2024). Furthermore, it is considered safe by regulatory authorities: the U.S. Food and Drug Administration (FDA) has classified *A. oryzae* as Generally Recognized As Safe (GRAS), and it has also been approved as a safe microorganism by the World Health Organization (WHO) (Seidler et al. 2024). This fungus has been widely utilized in the fields of food production, industry, medicine, and agriculture (García-Conde, Cerna-Chávez & Ochoa-Fuentes 2024; Seidler et al. 2024). Based on this species-level classification and its genetic identity to the safe neotype strain, the use of *A. oryzae* SAF1 for plant disease control presents a highly promising and potentially safe approach. However, since the safety of *A. oryzae* SAF1 in this study is inferred from phylogeny rather than direct toxicological evaluation, future studies involving specific toxicity testing of the SAF1 strain are warranted for future product registration and commercialization.

PLANT GROWTH-PROMOTING TRAITS OF *A. oryzae* SAF1

After 7 days of incubation, *A. oryzae* SAF1 produced IAA at a concentration of 19.50 µg/mL. This result is consistent with previous findings for this species, such as the report by Sanneboyina and Audipudi (2024) on the strain *A. oryzae* AVNF4. The ability to synthesize this phytohormone is a crucial mechanism for promoting plant growth, as it stimulates cell division and root elongation.

A. oryzae SAF1 produced ammonia at a concentration of 3.43 µmol/mL after 12 days of incubation. This finding supports previous reports on the ammonia-producing capabilities of *A. oryzae* (Sanneboyina & Audipudi 2024). Ammonia synthesis is considered a key plant growth-promoting trait, as its availability to the plant can enhance overall development, including shoot and root length, as well as fresh and dry biomass (Sonsiam et al. 2024).

The ability of *A. oryzae* SAF1 to grow and form a clear zone with a diameter of 29.44 ± 0.65 mm on a nitrogen-free medium indicates its potential for atmospheric nitrogen fixation (Figure 2(F)). This finding is significant, as phenotypic nitrogen-fixing capabilities have been observed in other fungal genera, such as *A. niger* (David et al. 2023) and *Trichoderma virens* (Cui et al. 2025), but have not previously been reported for *A. oryzae*. To the best of our knowledge, this is the first report of potential nitrogen-fixing ability in an *A. oryzae* strain. Regarding the genetic basis, while

nif genes have been detected in DNA extracted from certain fungal species like *Ustilago maydis*, a study has confirmed that these genes belong to intracellular nitrogen-fixing bacteria (e.g., *Bacillus* spp.) living symbiotically within the fungal cells (Ruiz-Herrera et al. 2015). Therefore, the exact biochemical pathway and the origin of this activity in *A. oryzae* SAF1 remain to be elucidated. Further research, including nitrogenase activity assays, screening for nitrogen-fixing genes (*nif* genes) and potential endosymbiotic bacteria, and definitive confirmation using $^{15}\text{N}_2$ isotope tracing, will be crucial to clarify this novel characteristic.

EFFICACY OF *A. oryzae* SAF1 ON DISEASE CONTROL AND PLANT GROWTH PROMOTION IN POT EXPERIMENTS

The efficacy of *A. oryzae* SAF1 as a biocontrol and plant growth-promoting agent was evaluated in pot experiments using two host plants: tomato, challenged with *Fol*, and chili, challenged with *C. gloeosporioides*. In the tomato trial, the application of *A. oryzae* SAF1 demonstrated significant efficacy in both disease control and growth promotion. For the biocontrol assessment, SAF1 treatment significantly reduced the Fusarium wilt DI from 67.50% in infected control plants (T3) to 15.00% (T4) ($p < 0.05$; Figure 4(A)). Furthermore, *A. oryzae* SAF1 demonstrated a significant plant growth-promoting effect across all measured parameters (Figure 4(B)–4(D)). Plants treated with SAF1 (T2 and T4) exhibited significantly greater ($p < 0.05$) shoot length, with an average of approximately 95 cm, compared to the untreated control (T1; 67.64 cm) and the pathogen-infected control (T3; 56.97 cm) (Figure 4(B)). This growth enhancement was also evident in biomass accumulation; the fresh weight of SAF1-treated plants (approx. 107 g) was substantially higher than that of the T1 (56.33 g) and T3 (41.65 g) groups (Figure 4(C)). A similar trend was observed for dry weight (Figure 4(D)). Interestingly, the absence of significant statistical differences in all growth parameters between the T2 (SAF1 alone) and T4 (SAF1 + *Fol*) treatments indicates that the growth-promoting efficacy of SAF1 remained fully effective even under pathogen pressure. In plant biology, the activation of defense mechanisms typically occurs at the expense of plant development, a well-documented evolutionary constraint known as the growth–defense trade-off where metabolic resources are shifted away from growth to ensure survival (Huot et al. 2014). However, our phenotypic data are consistent with the findings of Leibman-Markus et al. (2023), who demonstrated that beneficial fungal treatments could simultaneously promote tomato growth and enhance disease resistance, suggesting that the classical growth–defense trade-off can be mitigated or uncoupled under specific plant–microbe interactions. While the precise molecular and hormonal mechanisms underlying the SAF1-mediated responses remain to be

elucidated, the present results indicate that *A. oryzae* SAF1 exhibits dual potential as both a disease-suppressive and plant growth-promoting agent in tomato under pathogen challenge. Concurrently, tomato plants treated with *A. oryzae* SAF1 (T2 and T4) exhibited healthy green leaves and stems, as well as a larger size compared to T1 and T3. In contrast, tomato plants in T3 showed clear symptoms of Fusarium wilt, including leaf yellowing and wilting (Figure 4(E)). These findings are consistent with the study by Attia et al. (2022), who reported that another *Aspergillus* species, *A. fumigatus*, also

demonstrated significant potential in both controlling Fusarium wilt and promoting the growth of tomato plants.

Similarly, in the chili experiment, *A. oryzae* SAF1 was effective in mitigating anthracnose disease and enhancing plant growth. The DI for anthracnose was significantly lowered from 55.00% in the infected control (T3) to 7.50% in plants co-inoculated with SAF1 and the pathogen (T4) ($p < 0.05$; Figure 5(A)). In terms of growth promotion, *A. oryzae* SAF1 significantly enhanced all measured parameters under both healthy

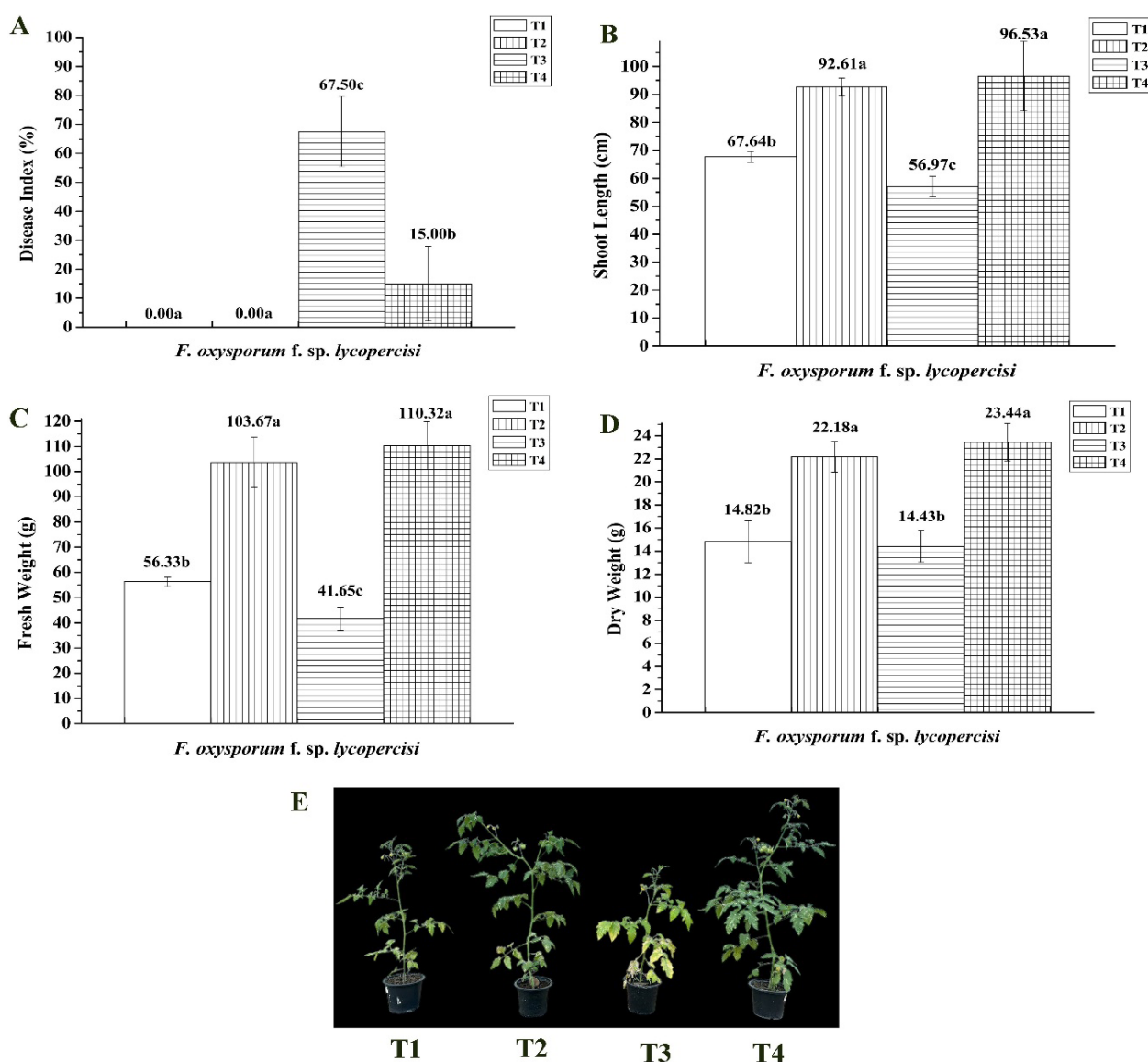


FIGURE 4. Efficacy of *A. oryzae* SAF1 in controlling Fusarium wilt disease (A) and promoting tomato growth: shoot length (B), fresh weight (C), dry weight (D), and growth under different treatments (E). Treatments are defined as follows: T1 (healthy control), T2 (SAF1 only), T3 (infected control, *Fol* only), and T4 (SAF1 + *Fol*). Data were recorded 60 days after planting. Bars represent mean values ($n = 10$), and error bars indicate standard deviation. Different letters indicate statistically significant differences at $p < 0.05$ based on the non-parametric Kruskal-Wallis test followed by the Mann-Whitney U test for (A), Welch's ANOVA followed by the Games-Howell post-hoc test for (B) and (C), and one-way ANOVA followed by Tukey's HSD test for (D).

and disease-pressure conditions (Figure 5(B)-5(D)). In healthy plants, the application of SAF1 (T2) resulted in greater shoot length (37.17 cm), fresh weight (39.11 g), and dry weight (5.86 g) compared to the healthy control (T1; shoot length 30.39 cm, fresh weight 13.42 g, dry weight 2.45 g). A more pronounced effect was observed under the pathogen challenge, where plants treated with SAF1 (T4) increased shoot length from 27.03 cm (T3) to 37.70 cm, fresh weight from 14.51 g to 28.79 g, and dry weight from 1.66 g to 4.40 g. Plants treated with *A. oryzae* SAF1 (T2 and T4) were visibly larger and healthier, exhibiting vibrant green leaves. In comparison,

the healthy control plants (T1) showed normal growth but were smaller than the SAF1-treated plants. Conversely, the infected control plants (T3) were clearly stunted and displayed severe anthracnose symptoms, including dark lesions on stems and leaf yellowing (Figure 5(E)). A noteworthy observation was that the T4 plants, despite being exposed to pathogen pressure, exhibited greater growth than the healthy control (T1). This observation suggests that *A. oryzae* SAF1 not only reduced disease severity but also contributed substantially to plant growth under both healthy and pathogen-challenged conditions. While such dual

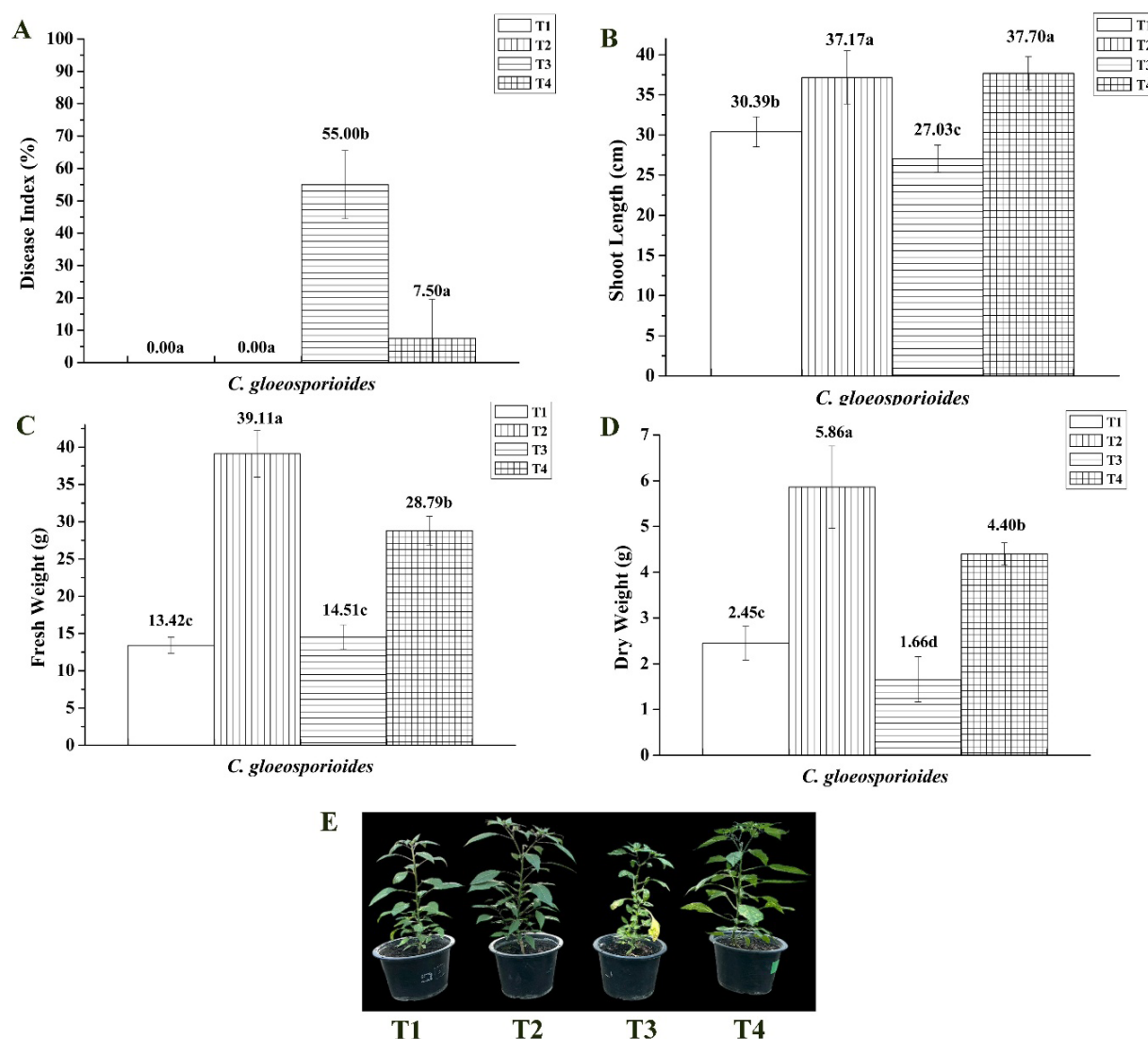


FIGURE 5. Efficacy of *A. oryzae* SAF1 in controlling anthracnose disease (A) and promoting chili growth: shoot length (B), fresh weight (C), dry weight (D), and growth under different treatments (E). Treatments are defined as follows: T1 (healthy control), T2 (SAF1 only), T3 (infected control, *C. gloeosporioides* only), and T4 (SAF1 + *C. gloeosporioides*). Data were recorded 60 days after planting. Bars represent mean values ($n = 10$), and error bars indicate standard deviation. Different letters indicate statistically significant differences at $p < 0.05$ based on the non-parametric Kruskal-Wallis test followed by the Mann-Whitney U test for (A), one-way ANOVA followed by Tukey's HSD test for (B), and Welch's ANOVA followed by the Games-Howell post-hoc test for (C) and (D)

effects have been documented for *Aspergillus* sp. NBP-08 against *C. capsici* (Naziya, Murali & Amruthesh 2020), other members of this genus are also known for their growth-promoting abilities. For instance, *A. tamarii* CY.OS 144 was shown to enhance chili growth through improvements in seed germination, biomass accumulation, and chlorophyll content (Sonsiam et al. 2024). However, to the best of our knowledge, no previous study has reported the simultaneous suppression of anthracnose disease and promotion of chili growth by *A. oryzae*. The present study therefore provides the first evidence supporting the dual role of *A. oryzae* SAF1 as both a biocontrol and plant growth-promoting fungus in chili under pot conditions.

The potential of *A. oryzae* SAF1 to control Fusarium wilt and anthracnose diseases can be attributed to several direct antagonistic mechanisms. The strain's ability to produce hydrolytic enzymes, such as chitinase, cellulase, lipase, and amylase, likely contributes to the degradation of fungal pathogen cell walls. This is complemented by its production of siderophores, which compete for essential iron, thereby limiting pathogen growth. These strategies, along with competition for space and nutrients, are well-documented antagonistic mechanisms in *Aspergillus* species (El-Maraghy, Tohamy & Hussein 2021; Wang et al. 2024). Moreover, the growth-promoting effects of *A. oryzae* SAF1 in both tomato and chili plants can be explained by its demonstrated abilities to produce ammonia, synthesize IAA, and fix nitrogen. The production of ammonia and IAA are well-established growth-promotion mechanisms in *Aspergillus* species, as reviewed by Adedayo and Babalola (2023). Nitrogen fixation by fungi is known to play a role in increasing nitrogen availability for plants, thereby supporting their growth (Cui et al. 2025; David et al. 2023). Collectively, the combination of these *in vitro* biocontrol and growth-promoting traits provides a plausible mechanistic basis for the disease suppression and enhanced plant growth observed in our pot experiments, highlighting the potential of *A. oryzae* SAF1 as a biocontrol and plant growth-promoting agent.

CONCLUSIONS

Aspergillus oryzae SAF1, a potentially safe fungal strain, demonstrates significant potential as a dual-action biocontrol and plant growth-promoting agent. It effectively inhibited key phytopathogenic fungi, including *Fol* and *Colletotrichum* spp., through direct biocontrol mechanisms such as the production of hydrolytic enzymes and siderophores. In addition, the strain promoted plant growth through the production of ammonia and IAA. This study also provides the first report suggesting a potential for nitrogen fixation in *A. oryzae*, a trait that complements its other growth-promoting mechanisms. These combined abilities resulted in significant disease control and growth

enhancement for tomato and chili in pot experiments, providing the first evidence of *A. oryzae* simultaneously suppressing anthracnose disease and promoting plant growth in chili. Therefore, *A. oryzae* SAF1 is a highly promising candidate for developing sustainable agricultural solutions that can reduce dependence on chemical inputs. Further research involving specific toxicity testing and the elucidation of the underlying mechanisms or origins of nitrogen fixation will be critical for product registration, while field trials remain essential to validate its efficacy under more complex environmental conditions.

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