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Research Article

Protective role of endophytic fungi and salicylic acid as therapeutic nutrients to improve immune responses of tomato plants against fusarial wilt disease

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Abstract

The exacerbation of climatic changes helped to increase the risk of plant diseases in the world. Based on this concept, we suggest Endophytic fungi (EF) and Salicylic acid (SA) alternatives to reduce the spread of *Fusarium* wilt disease, which is one of the most important agricultural crops globally. Therefore, this study aims to isolate endophytic fungi and test their ability to stimulate plant resistance against *Fusarium* wilt disease and study the possibility of combining these isolates with salicylic acid as a therapeutic nutrient for tomato plants. Two fungal endophytes were isolated from *Vigna unguiculata* seeds (VUS) and identified as *Aspergillus oryzae* and *Aspergillus tubingensis* according to morphological and molecular identification. Under pot circumstances, the influence of these two fungal endophytes as tomato fusarial wilt depressants and as plant stimulants was evaluated. The combination of *A. oryzae* and *A. tubingensis* exhibited a simultaneous efficacy of 80% in conferring protection against *Fusarium* wilt, while simultaneously resulting in a notable decreasing diseases index to 16.66%. Treatment of infected plants with *A. oryzae* and *A. tubingensis* (single or mixed) and salicylic acid not only deterioration influence of *Fusarium* wilt on plant fitness, but also showed a significant improvement in the levels of photosynthetic pigments, sugars, proteins and phenols, and enhancement in the activity of antioxidant enzymes. The analysis of variability among endophytic fungi revealed the presence of 17 distinct bands exhibiting polymorphism, accounting for approximately 35.294% of the observed genetic variation. In conclusion, endophytic *A. oryzae* and *A. tubingensis* work well as stimuli for tomato plant growth and as a means of boosting the plants' resistance to *Fusarium* wilt. Further, *A. oryzae* and *A. tubingensis* act synergistically with SA toward improving plant growth and fusarial wilt disease resistance.

Keywords: biocontrol; fungal endophytes; antifungal activity; induced systemic resistance

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Introduction

Plant fungal diseases are a major problem for plant production around the world, as they can result in full or partial crop loss (Loha *et al*., 2018; Hashem *et al*., 2021). All crops have been subjected to infections, particularly tomatoes, which yearly cause about 70-95% output losses (Lukyanenko, 1991; Quesada-Ocampo *et al*., 2023). Fungal plant diseases including pre and post-harvest led to huge harmful effects on food and economic values (Khalil *et al*., 2019). Thus, it is crucial to develop more effective management methods that are economically affordable and environmentally safe (Attia *et al*., 2016). The stimulating effect on plant biomass and the repressive impact of diseases are essential for biological control (Khattab *et al*., 2022). In contrast to fungicides, natural management of *Fusarium* wilt uses biotic agents that have the potential for reducing the deleterious effects of the disease on crops (Abdelaziz *et al*., 2022). Therapeutic feeding is a nutrition that consists of providing nutrients and fertilizers to the plant that activate and stimulate physiological processes and help increase the plant's ability to confront challenges and hazards, as well as minimize some diseases or side effects linked with those diseases (Attia *et al*., 2023).

Fungal endophytes are organisms live in tissues beneath the epidermal cell layers and have no obvious negative effects on the host plant (Yu *et al*., 2010). Based on its ability to create antimicrobials, antioxidants, minerals, vitamins, and growth regulators (Hashem *et al*., 2023; Sharaf *et al*., 2022). Fungal endophytes were reported to enhance nutrient uptake and assimilation in the host plant thus researchers have recently inclined to employ the filtrate of fungal endophytes to supply the plant with nutrients and lessen the severity of disease infection (Chaudhary *et al*., 2022). Fungal endophytes are recognized to generate a wide group of secondary metabolites, involving alkaloids, terpenoids, phenolics, and peptide (Rai *et al*., 2021; Khalil *et al*., 2021). The role of Fungal endophytes in supporting the plants to environmental stresses, such as drought, high temperatures, or nutrient deficiencies has been proven (Badawy *et al*., 2021). Further, fungal endophytes can contribute to the plant defense against pathogens and herbivores by producing defense related compounds that deter or inhibit the pathogen growth and disease development (Verma *et al*., 2022).

Numerous investigations have shown that salicylic acid is one of the main hormonal signals dictating the fate of plants under stress (Farrag *et al*., 2021; Omidi *et al*., 2022). In this respect, it has been shown that salicylic acid contributes significantly to plant-induced resistance and is linked to defense-related reactions in plants against infection by different pathogens (Xie *et al*., 2018). Induced resistance, a physiological defense mechanism against plant fungal infections, is activated by specific external factors, inducers, and involves increased production of phytoalexins and a variety of proteins (Patel *et al*., 2020). In fact, resistance is a salicylic acid-dependent signaling pathway that is usually connected with systemic expression of pathogenesis-related protein genes and other potential defenses (Shine *et al*., 2019).

Vigna unguiculata belongs the leguminous family Fabaceae and considered as one of the most important vegetable crops that are grown for export or local production (Guterres *et al*., 2022). The dry VUS is a very rich source of carbohydrates, proteins and calcium, as well as phosphorus, iron, thiamine, riboflavin and niacin (Kebede and Bekeko, 2020). By establishing an environment that is favorable for seed germination and conservation through the production of various metabolites, seed-associated fungi aid in seed germination and conservation. In this study, endophytic fungi from VUS will be isolated and used singly or in combination to improve tomato plant development and establish resistance to *Fusarium* wilt disease. It also attempts to increase tomato plant resistance to *Fusarium* wilt disease by applying these fungal isolates with salicylic acid.

Materials and Methods

Isolation of fungal endophytes

Healthy VUS were initially washed by sterile distilled water, subsequently dipped in 70% ethanol for sixty seconds, then in 4% NaOCl for the same time, and then thoroughly rinsed several times. Using sterile filter sheets to dry the seeds after superficial sterilization, they were then cut into 0.25 X 0.25 centimeters. Then, all sterilizes segments were plated on to surface of PDA plates and incubated for 21 days at 27 ° C ± 2 ° C, and subsequently examined every day with a stereomicroscope (Khalil *et al*., 2021).

Identification of fungal isolates

Fungal isolates were identified based on their morphological characters as well as molecular. Both microscopic descriptions of the fungal vegetative and reproductive structures as well as macroscopic morphological characteristics such as color, texture, and colony diameter have been taken (Moubasher and Moustafa, 1970; Abdel-Azeem, 2010). Also, fungal isolates were identified genetically. The primer sequences used in this procedure were ITS1-F (forward) and ITS2-R (reverse) (50-TCCTCCGCTTATTGATATGC-30). The produced sequence was sent to the NCBI database's BLAST program to be looked for phylogenetic sequences that were remarkably similar to it. The Mega 5.0 program's neighbor-joining approach was used to create the tree of phylogeny.

Source of pathogen

F. oxysporum f. sp. Lycopersici RCMB008001 was obtained from Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University. The pathogenic fungus inoculum was made by combining the contents of five pure cultures that had been grown on PDAs at 24 °C for six days.

Pots trial

Tomato 023 uniform seedlings (were obtained from the Agricultural Research Center in Giza, Egypt) that were three weeks old were transplanted into plastic pots with a diameter of 30 cm that had been containing mixture of sand and clay (1: 3 W/W) (Ec 1.8 $dS.m^{-1}$, pH 6.4), that contained a total of 6 kg. This commercial cultivar was chosen because it is commonly used in the agriculture areas of Egypt for its good adaptation and highly yields. During winter season, pots were kept in the greenhouse having 22/18 °C day/night temperature and 70-85% relative humidity The pots were arranged in a completely randomized design with six replicates as the following: (T1) Healthy control: Tomato seedlings grown in newly sterilized soil ; (T2) Control infected: Growing tomato seedlings in *Fusarium*-inoculated, sterile soil; (T3) Infected seedlings and treated with salicylic acid-treated; (T4) Infected seedlings and treated with *A. oryzae*; (T5) Infected seedlings and treated with *A. tubingensis*; (T6) Infected seedlings and treated with (*A. oryzae* and *A. tubingensis*); (T7) Healthy seedlings and treated with salicylic acid; (T8) Healthy seedlings and treated with *A. oryzae*; (T9) Healthy seedlings and treated with *A. tubingensis*; (T10) Healthy seedlings and treated with (*A. oryzae* and *A. tubingensis*).

Disease index

In the current study, the method used by Abdelaziz *et al*. (2022) was used for evaluation the disease index and plant protection. Disease signs appeared 45 days after transplanting. It should be noted that the percent disease index (PDI) was calculated using a five-grade scale according to the formula; PDI = (1n1+ 2n2+ 3n3+ 4n4)100/4nt, where n1-;n4 is the number of plants in each class and nt is the total number of plants evaluated. Furthermore, the following formula was used to calculate % Protection (P percent): P percent = A - B/A 100%, where A is the PDI in the infected control.

Resistance indicators

Morphological characters and photosynthetic pigments

Morphological characters and biochemical of all plant trials were detailed at 70 days after planting. The lengths of the shoots were measured at random from above the soil surface to the end of the plant growing tip and recorded in cm. Root lengths were measured from the soil surface to the end of the root tip and recorded in cm according to Attia *et al*. (2021). Three plants from each treatment were counted to determine the number of leaves. The quantification of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids in fresh tomato leaves was conducted according to the methodology described by Vernon and Ke (1966). Fresh tomato leaves (0.5 g) were used to extract photosynthetic pigments using 50 mL of acetone (80%). The extract was then filtered, and the green colour was measured at 665, 649, and 470 nm.

Biochemical

Total carbohydrates

The quantification of soluble carbohydrates in the shoot was determined using the methodology outlined in the study conducted by Irigoyen *et al*. (1992). After dilution with 5 mL of 30% trichloroacetic acid (TCA) and 2.5 mL of 2% phenol, the 0.5 g dried shoots were filtered through filter paper. One millilitre of the filtrate was then subjected to 2 millilitres of anthrone reagent (2 grams of anthrone per liter of 95% H_2SO_4). The blue-green colour that was created was measured at 620 nm.

Total proteins

The Lowry method was employed to evaluate the overall concentration of soluble proteins (Lowry *et al*., 1951) The dried tomato shoots were extracted using 10 mL of distilled H_2O and 5 mL of 2% phenol. This extract was diluted 1:3 v/v in Folin's reagent and mixed with 1 mL of alkaline reagent (5 mL of 2% Na_2CO_3 prepared in 0.1N NaOH and 1 mL of 0.5% $CuSO_4$ prepared in 1% potassium sodium tartrate). At 750 nm, there was a noticeable colour shift after 30 minutes.

Total phenols

The quantification of shoot phenol content was conducted utilizing the procedure established by Danil and George (1972). For one night, 0.5 g of dried tomato shoots were extracted in 5 mL of 80% ethanol. And repeat this three times, then added 80% ethanol to fill to a 50 ml capacity. For three minutes, 0.5 mL of the extract and 0.5 mL of Folin's reagent were well combined and agitated. After that, 1 mL of saturated sodium carbonate solution and 3 mL of distilled water were added and well combined. After an hour, the blue colour was observed at 725 nm.

Antioxidant enzymes

In a centrifuge, one g of fresh tomato shoots were homogenized with 10 ml of phosphate buffer pH 6.8, and the mixture was then centrifuged at 2 °C for 20 minutes at 20,000 rpm. The source of the enzymes was determined to be the clear supernatant that contained the enzymes. The assay for peroxidase activity (POD) was carried out following the methodology outlined by Bergmeyer (1974). The enzymatic activity of polyphenol oxidase (PPO) was assessed using the methodology used by Matta and Dimond (1963). The assessment of superoxide dismutase (SOD) activity was conducted utilizing the methodology established by Marklund and Marklund (1974). The assessment of catalase (CAT) activity was conducted in accordance with the established protocol outlined by Aebi (1984).

SDS-PAGE

The protein fingerprint of tomato leaves was subjected to analysis utilizing SDS-PAGE, employing the methodology established by Laemmli (1970). The Gel Doc VILBER LOURMAT methodology was employed to assess and examine gels. The banding structure was recorded while the gels were saturated, and the number

of the band was identified in each gel lane, computed, and correlated with the others. Band strength and band quantity changes were quantified using the Helena Densitometer Model Junior 24.

Statistical analysis

Duncan's multiple rang test and the least significant difference (L.S.D) at a 5% level of probability were used to determine the differences between means. This analysis was performed using Co-state software subsequent to subjecting the experimental data to one-way analysis of variance (ANOVA).

Results

Identification of endophytic fungal isolates

Two fungal isolates were isolated and identified morphologically and genetically as *A. tubingensis* and *A. oryzae*. *Aspergillus oryzae* morphology was the following; on PDA medium, colonies fast growth with dark yellowish green powdery reaching 50 mm diameter in 5 days at 28 °C with creamy (Figure 1A), light brown reverse (Figure 1B), vesicles globose to sub-globose shape, metula and phialides long covered 75% of the head; conidia were spherical (Figure 1C).

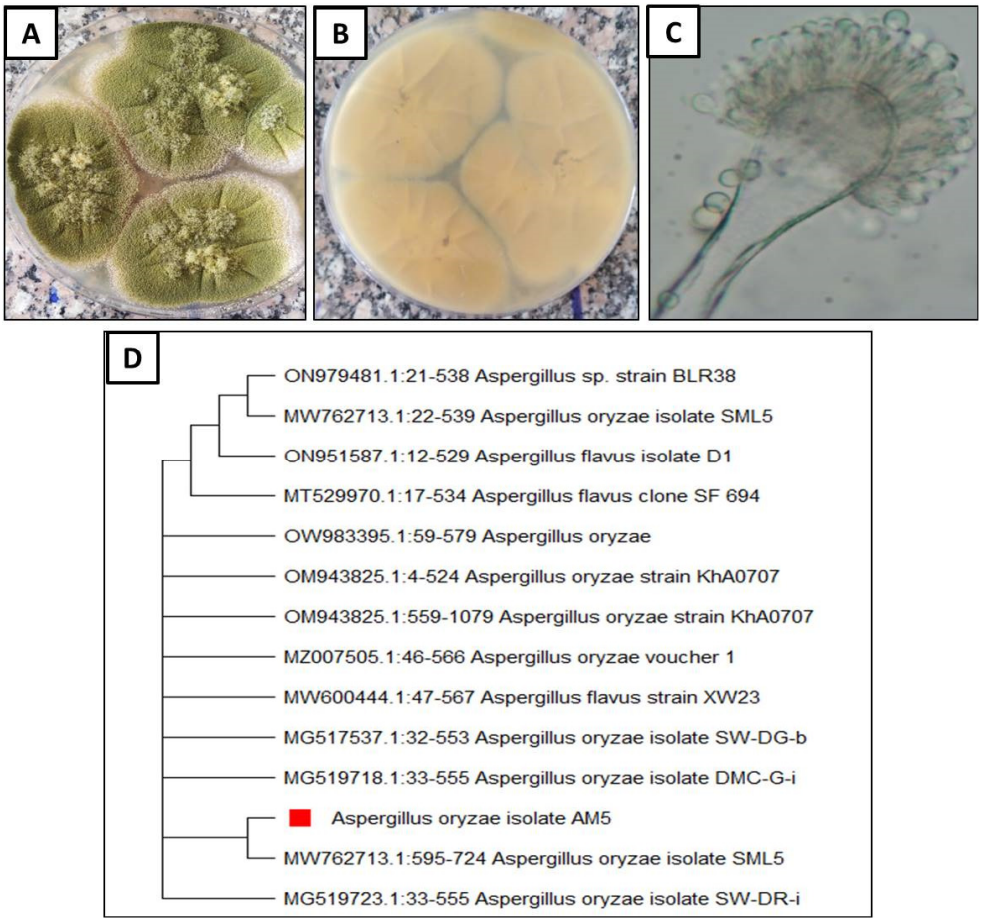

Figure 1. Morphological and molecular identification of *A. oryzae* (A-D): A) Surface colony on PDA; B-Reverse color; C) Conidiophore and conidia; D) Phylogenetic tree

A. tubingensis morphology was a colony on a PDA after five days of frenzied development at 28°C, the mycelium is 60 mm in diameter (Figure 2A), reverse deep black (Figure 2B), vesicles are globose in shape, biserrate, metula and phialides long covered all the head; conidia were spherical (Figure 2C). Additionally, molecular identification was carried out to confirm routine identification; two fungal strains sequences of *A. tubingensis* and *A. oryzae*. The phylogenetic analysis of these fungal strains revealed approximately 98 identify for both *Aspergillus tubingensis* (Figure 1D) and *Aspergillus oryzae* (Figure 2D) with ITS sequences of rRNA genes of related species using BLAST programs.

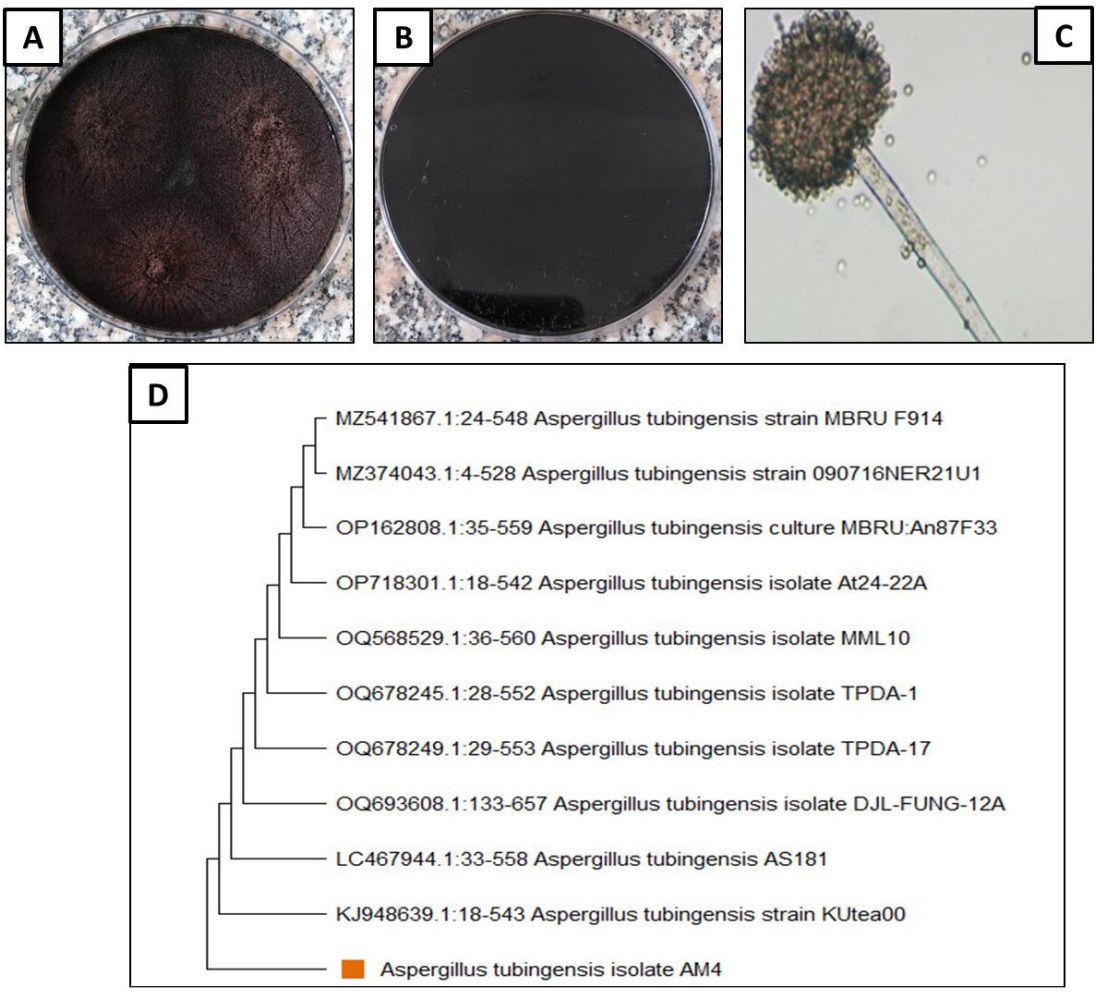

Figure 2. Morphological and molecular identification of *A. tubingensis* (A-D): A) Surface colony on PDA; B- Reverse color; C) Conidiophore and conidia; D) Phylogenetic tree

Effect of A. oryzae and A. tubingensis on disease index

The data in Table 1 showed that, as compared to infected plants, treatment with tested isolates or salicylic acid substantially decreases the incidence of disease indications. When comparing the infected control plants (83.33%) to the experimental groups, it was observed that the combined co-application of *A. oryzae* and *A. tubingensis* led to decrease in the percentage of disease (16.66%). This was followed by the application of *A. oryzae* (25%), salicylic acid (33.33%), and finally *A. tubingensis* (45.83%). Application mixture of (*A. oryzae* and *A. tubingensis*) showed a synergistic impact that significantly reduced Fusarium wilt disease in tomato plants by 80%.

Table 1. Effect of *A. oryzae* and *A. tubingensis* on disease index

Treatment	Classes of disease symptoms					DI (%)	Protection (%)
	0	1	2	3	4		
Healthy control	6	0	0	0	0	0	-
Infected control	0	0	1	2	3	83.33	0
Infected + salicylic acid	2	2	0	2	0	33.33	60
Infected + *A. oryzae*	2	2	2	0	0	25	69.99
Infected + *A. tubingensis*	1	3	0	0	2	45.83	44.99
Infected + *A. oryzae,* and *A. tubingensis*	3	2	1	0	0	16.66	80

Effect of tested elicitors on morphological indicators

The results depicted in Figure 3 indicate that *F. oxysporum* exerted a negative effect on all aspects of vegetative growth. *F. oxysporum* exhibited a notable reduction in shoot length, resulting in a decrease of approximately 40%. Similarly, the presence of *F. oxysporum* had a significant impact on root length, causing a reduction of approximately 51.17%. Furthermore, the number of laterals experienced a decline of approximately 40% in the presence of *F. oxysporum*. The utilization of *A. oryzae* and *A. tubingensis* resulted in a notable enhancement in shoot length, with increases of 62.67% and 51.86% observed, respectively. Similarly, root length exhibited substantial growth, with increments of 84.85% and 79.9% for *A. oryzae* and *A. tubingensis*, respectively. Furthermore, the application of these fungi led to a significant rise in the number of laterals, with increments of 105% and 94.5% observed for *A. oryzae* and *A. tubingensis*, respectively. The observed morphological indicators exhibited significant enhancements in the healthy plant subjected to all the tested elicitors. The use combination of (*A. oryzae* and *A. tubingensis*) showed greatly improved shoot length, root length, and number of leaves. The results in Figure 3 indicated that salicylic acid improved plant morphological characteristics, both in healthy and infected plants.

Photosynthetic pigments

As depicted in the data presented in Figure 4, the infection caused by *F. oxysporum* resulted in a decrease of ch a by 45.6% and ch b by 43.6%. Conversely, there was a significant increase in carotenoid content, which rose by 144.8%. All the elicitors evaluated demonstrated enhanced effects on the photosynthetic pigments ch a, ch b, and carotenoids. Regarding the impact assessed elicitors, on the infected plants with *F. oxysporum*, it was observed that the combination treatment of (*A. oryzae* and *A. tubingensis*) exhibited a remarkably substantial rise in the levels of ch a and ch b by (74.5% and 63.4), respectively. The results in Figure 4 indicated that salicylic acid improved ch a, ch b as well as carotenoids, both in healthy and infected plants. Also, results revealed that the contents of carotenoids were higher than healthy control. Additionally, the results showed that the use of the evaluated elicitors enhanced the amounts of carotenoids in both healthy and *Fusarium*-infected plants. But when compared to healthy control plants, healthy tomato plants treated with all of the test elicitors displayed higher levels of photosynthetic pigments (chlorophyll and carotenoids).

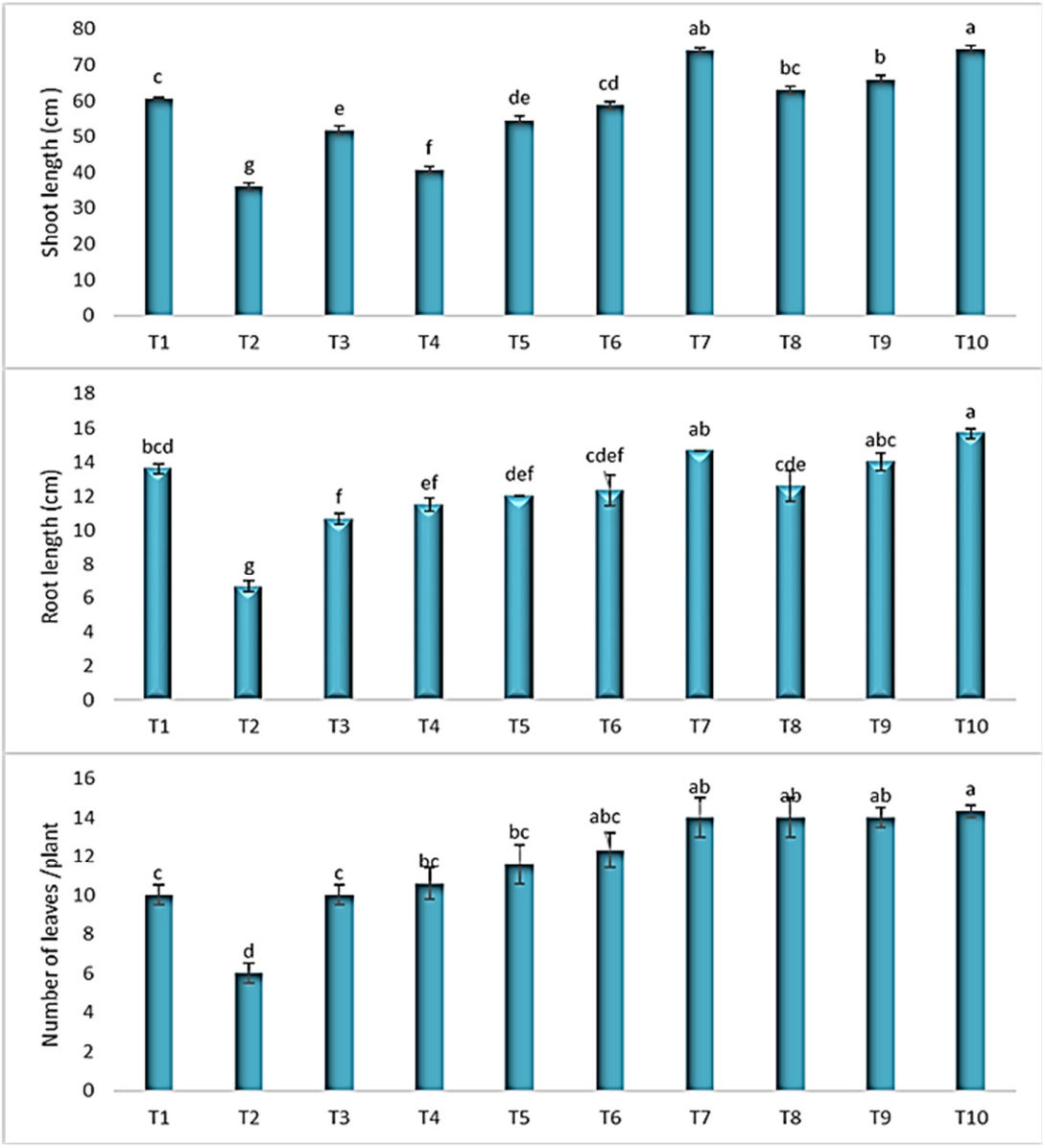

Figure 3. The effect of studied elicitors on A) shoot length, B) root length, and C) number of leaves of tomato plant

Data presented as means ± SD (n=3). Data LSD test at P ≤ 0.05. (T1) Healthy control; (T2) Control infected; (T3) Infected + salicylic acid; (T4) Infected + *A. oryzae*; (T5) Infected + *A. tubingensis*; (T6) Infected + (*A. oryzae* and *A. tubingensis*); (T7) Healthy + salicylic acid; (T8) Healthy + *A. oryzae*; (T9) Healthy + *A. tubingensis*; (T10) Healthy + (*A. oryzae* and *A. tubingensis*).

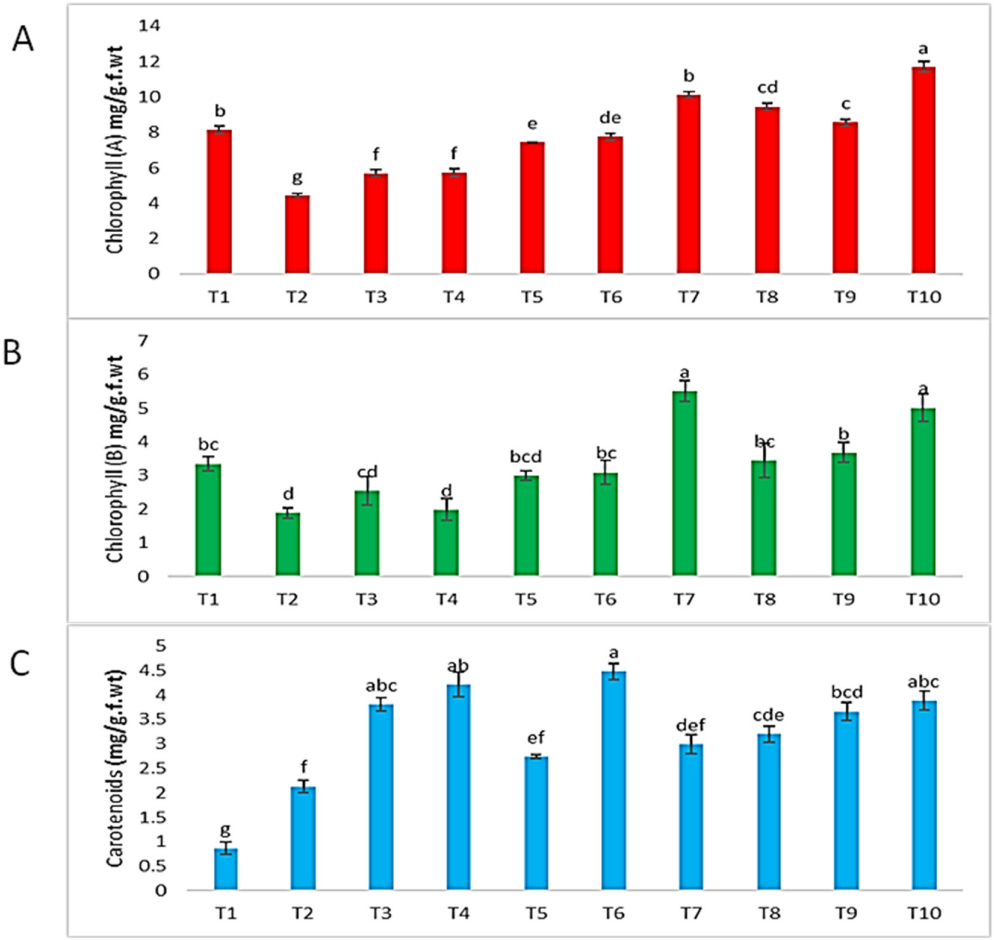

Figure 4. The effect of studied elicitors on A) cholorophyll a, B) cholorophyll b, and C) carotenoids of tomato plant

Data presented as means ± SD (n=3). Data LSD test at P ≤ 0.05. ((T1) Healthy control; (T2) Control infected; (T3) Infected + salicylic acid; (T4) Infected + *A. oryzae*; (T5) Infected + *A. tubingensis*; (T6) Infected + (*A. oryzae* and *A. tubingensis*); (T7) Healthy + salicylic acid; (T8) Healthy + *A. oryzae*; (T9) Healthy + *A. tubingensis*; (T10) Healthy + (*A. oryzae* and *A. tubingensis*)).

Biochemical resistance indicators

The observations illustrated in Figure 5, in comparison to tomato plants in a healthy control group, the infected plants exhibited a reduction in their carbohydrate content by 32.31%. A notable augmentation of protein levels, amounting to 8.38%, was observed in the infected plants. Moreover, the application of all the examined elicitors on tomato seedlings, whether they were in a healthy or infected with *Fusarium*, resulted in a notable increase of soluble sugars and soluble proteins. When comparing to control plants, the combination of *A. oryzae* and *A. tubingensis* showed the greatest rise in soluble sugar and soluble protein contents. The results in Figure 5 indicated that salicylic acid improved total soluble carbohydrate and total soluble protein, both in healthy and infected plants. Tomato plants infected with Fusarium exhibited a significant increase in total phenol levels, amounting to a 69.52% rise in comparison to a control group of healthy plants. As well, the use of elicitors on plants exposed to *Fusarium*-infection resulted in a significant increase in the amounts of total phenols in (*A. oryzae* and *A. tubingensis*), *A. oryzae*, *A. tubingensis*, and salicylic acid, with increases of 127.5%, 107.3%, 92.69%, and 70.78%, respectively (Figure 5). Also, application of SA led to increase in total phenols in healthy and infected plants. It was shown that all treatments led to a noticeably higher overall amount of

phenolic compounds when compared to the healthy control plants and the tomato plants that had received the treatment.

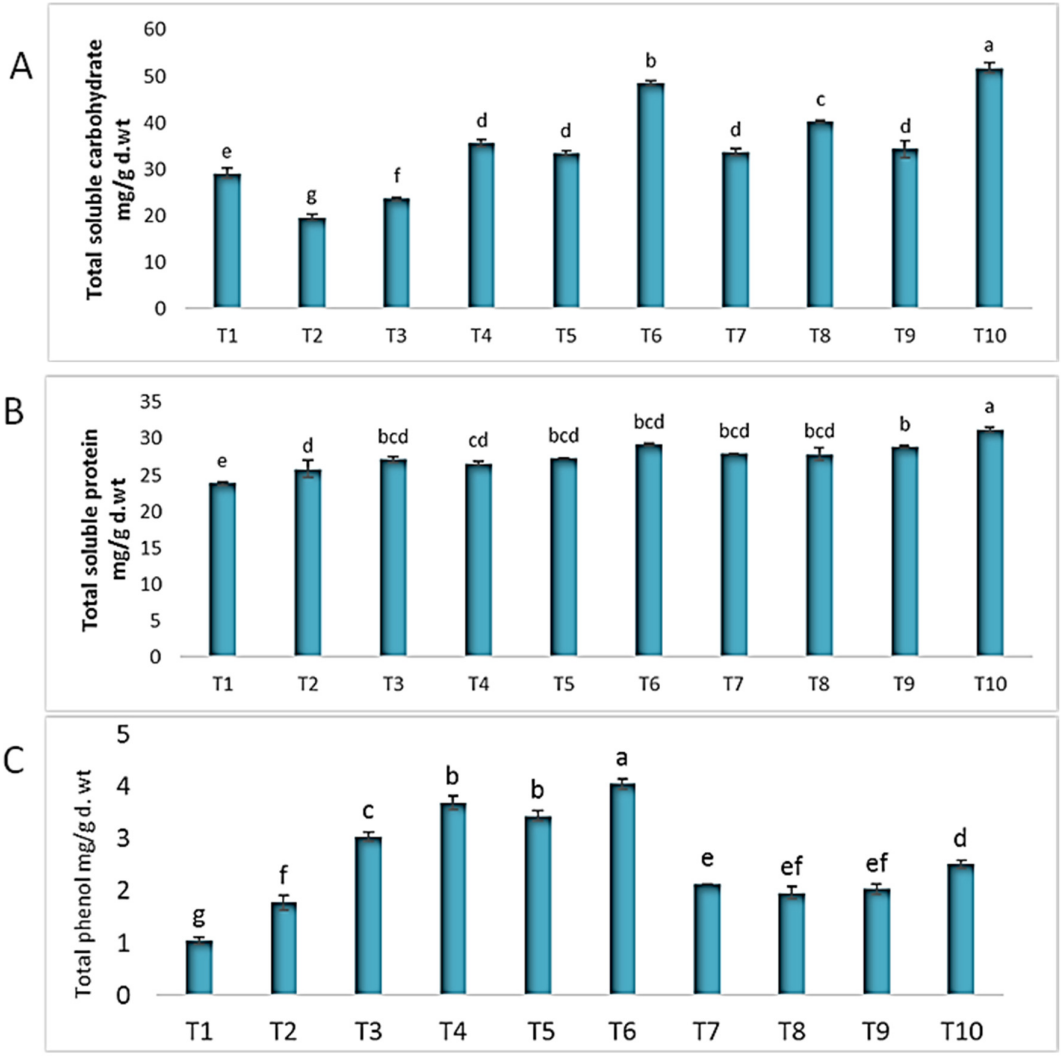

Figure 5. The effect of studied elicitors on A) total soluble carbohydrates, B) total soluble proteins, and C) total phenols of tomato plant

Data presented as means ± SD (n=3). Data LSD test at P ≤ 0.05. ((T1) Healthy control; (T2) Control infected; (T3) Infected + salicylic acid; (T4) Infected + *A. oryzae*; (T5) Infected + *A. tubingensis*; (T6) Infected + (*A. oryzae* and *A. tubingensis*); (T7) Healthy + salicylic acid; (T8) Healthy + *A. oryzae*; (T9) Healthy + *A. tubingensis*; (T10) Healthy + (*A. oryzae* and *A. tubingensis*)).

Oxidative enzymes activity

In this study, the mean activities of PPO, POD and SOD in the tested tomato plants were estimated. Our aim was to gain a more comprehensive understanding of the diverse defense-related enzymes present. When juxtaposed with the untreated control plants, it was observed that tomato plants afflicted with *Fusarium* infection exhibited heightened levels of oxidative enzyme activity. Furthermore, it has been revealed that all treatments exhibited a substantial enhancement in the activity of antioxidant enzymes within the population of thriving plant organisms. Moreover, compared to plants that were only exposed to infection, application of (*A. oryzae* and *A. tubingensis*), showed a clear increase in the activity of the antioxidant enzymes Superoxide

Dismutase (SOD), Polyphenol Oxidase (PPO), and Peroxidase (POD) (Figure 6). Also, results showed that salicylic acid improved the activity of the antioxidant enzymes SOD, PPO and POD in both healthy and infected plants.

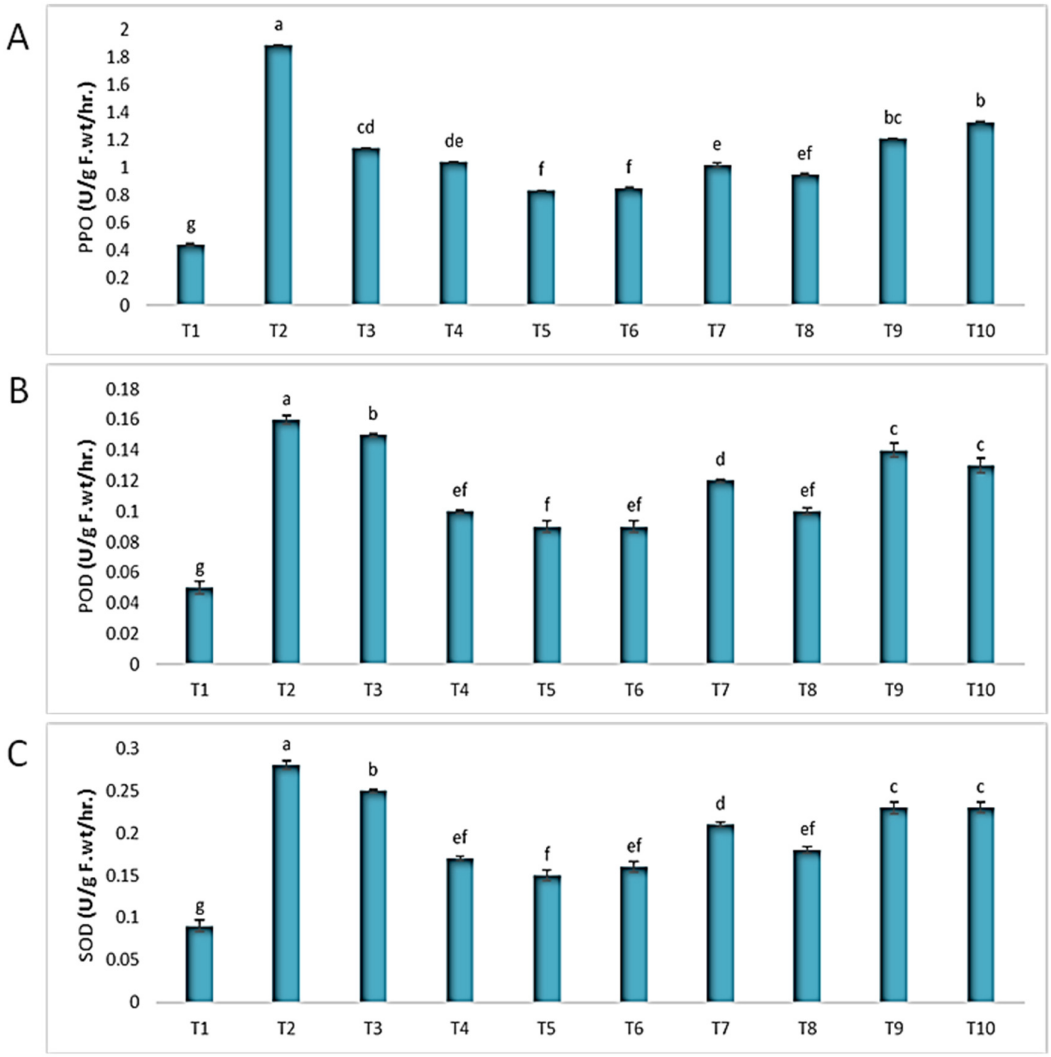

Figure 6. The effect of studied elicitors on A) PPO, B) POD and C) SOD of tomato plant
Data presented as means ± SD (n=3). Data LSD test at P ≤ 0.05. ((T1) Healthy control; (T2) Control infected; (T3) Infected + salicylic acid; (T4) Infected + *A. oryzae*; (T5) Infected + *A. tubingensis*; (T6) Infected + (*A. oryzae* and *A. tubingensis*); (T7) Healthy + salicylic acid; (T8) Healthy + *A. oryzae*; (T9) Healthy + *A. tubingensis*; (T10) Healthy + (*A. oryzae* and *A. tubingensis*)).

Genetic markers

The SDS-PAGE analysis revealed discernible modifications in the protein patterns observed between the elicitors and the *Fusarium* infection within the protein profile. Furthermore, an increased proportion of polymorphism was discerned in plants that underwent elicitor treatment, as indicated by the presence of variations in the quantity of bands and the molecular weight of individual proteins. The aforementioned observations are elucidated in Figure 7. Our observations revealed the existence of proteins exhibiting molecular weights of 26, 32, and 37.252 kilodaltons (PR3-Chitinase). These proteins have been categorized as pathogenesis-related proteins and are known to play a role in plant defense mechanisms owing to their anti-

pathogenic properties. The presence of these induced proteins was observed across all plant specimens subjected to elicitor treatment.

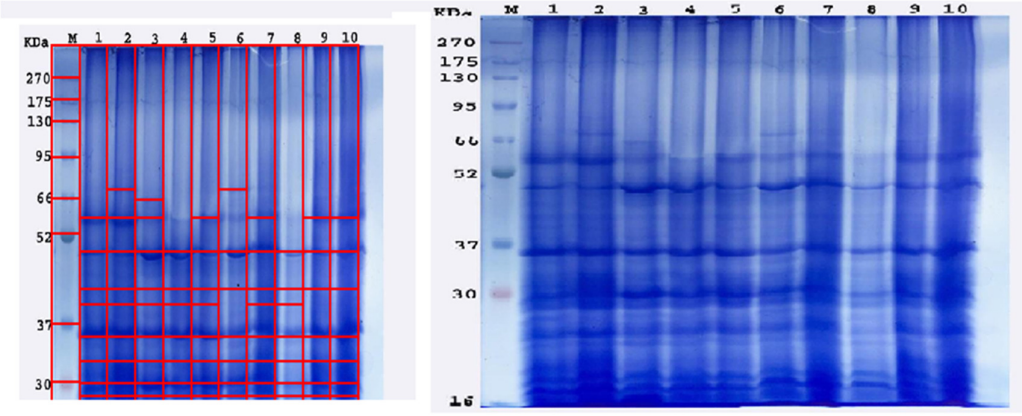

Figure 7. The effect of studied elicitors on protein fractions of tomato plant using SDS PAGE ((1) Healthy control; (2) Control infected; (3) Infected + salicylic acid; (4) Infected + *A. oryzae*; (5) Infected + *A. tubingensis*; (6) Infected + (*A. oryzae* and *A. tubingensis*); (7) Healthy + salicylic acid; (8) Healthy + *A. oryzae*; (9) Healthy + *A. tubingensis*; (10) Healthy + (*A. oryzae* and *A. tubingensis*)).

Discussion

Plant resistance may be enhanced by the exogenous application of biotic elicitors through the use of fungal endophytes with antimicrobial properties. The seeds of Healthy *V. unguiculata* were discovered to possess a diverse array of bioactive compounds, totaling 49 in number (Razgonova *et al*., 2022). *A. tubingensis* and *A. oryzae*, two endophytic fungi isolated from healthy *V. unguiculata* seeds, then identified for this purpose. Our data support their conclusion that the endophytic fungus Ascomycota were the most prevalent in Fabaceae family (Kinge *et al*., 2022).

This study examined the effects of endophytic fungi (*A. tubingensis* and *A. oryzae*) and salicylic acid on both infected and healthy tomatoes. The results showed an 80% increase in protection against Fusarium wilt due to the significant boost of the tomato defense mechanism following the integration of endophytic fungi. It has also been noted that salicylic acid increases tomato resistance to *Fusarium* wilt and has high percentage of inhibition against *F. oxysporum* (Attia *et al*., 2016). Similarly, Kriaa *et al*. (2015) reported that *A. tubingensis* exhibited antifungal properties against *Fusarium*, it has been used as a novel bio fungicide to combat plant *Fusarium* infections. It has been reported that *A. tubingensis* has been employed as a possible agent for managing *Fusarium* wilt because of its significant anti-*Fusarium* wilt action brought on by a unique detoxification of the mycotoxin fusaric acid (Crutcher *et al*., 2014). By producing phenolic and flavonoid chemicals, endophytic *A. oryzae* also showed these antifungal properties (Qiu *et al*., 2010).

The findings presented in the current study demonstrate that the presence of *Fusarium* wilt exerts a detrimental influence on the morphological attributes of tomato plants. The observed diminishment in all growth characteristics could potentially be attributed to alterations in the accessibility or dispersion of growth-regulating hormones, as elucidated by Farrag *et al*. (2017); El-Batal *et al*. (2023). The data shown in the present study clearly demonstrate that, in comparison to the control group, the application of the combination of *A. tubingensis* and *A. oryzae* led to a notable augmentation in shoot length, root length, and lateral number.

Results in Figure 3 showed that the failure of infected plants to produce photosynthetic pigments. This may be caused by an oxidative stress situation that damages chlorophyll a, which results in a reduction or inhibition of photosynthesis (Hashem *et al*., 2023). On the other hand, application of endophytic fungi, especially with the combination, improved photosynthetic pigments. The concept that endophytic fungi can improve plant performance and photosynthetic capability explains these findings (Abdelaziz *et al*., 2022). As a result of several changes in chloroplast form, location, and function during infection, correlations between fungal disease and carotenoid or chlorophyll concentrations have been established (Kretschmer *et al*., 2020).

The results of the study show a significant increase in the protein content of tomatoes infected with *Fusarium* fungus. In this regard, Nafie (2003) showed that the pathogenic *F. oxysporum* instigates the intricate process of nitrogenous constituent biosynthesis within plants, specifically during the acute stage of infection. The plants that were infected displayed a reduction in their photosynthetic capacity, accompanied by an increase in their respiration rate, resulting in a decline in the availability of soluble carbohydrates. The findings of this investigation have unveiled that the application of the elicitors being studied has exerted a significant impact on the levels of total soluble sugar and soluble protein. The observed modifications in osmolytes possess the inherent capacity to serve as a discernible marker of resistance. Additionally, these fungi exhibit the capacity to solubilize phosphate, thereby enhancing nutrient uptake from the root rhizosphere (Attia *et al*., 2022). Consequently, these findings contribute to the enhancement of plant health and establish the potential of these fungi as a biofertilizer (Rana *et al*., 2020).

Phenolic compounds exhibit a crucial function in preventing the spread of wilt diseases through their ability to synthesize numerous secondary metabolites, which are known to be involved in host defense mechanisms. Additionally, these compounds help mitigate the pathogen toxicity while simultaneously enhancing the host defense pathways. Moreover, phenolic compounds exhibit the capacity to stabilize cellular membranes through the restriction of membrane fluidity. This, in turn, reduces the permeability of free radicals across the membranes and subsequently mitigates membrane peroxidation (Kubalt, 2016).

In this context, our findings demonstrated that plants subjected to *Fusarium* infection had considerably higher activity levels of antioxidant enzymes. The plant displayed various defense mechanisms against infection, upping the activity of specific antioxidant enzymes to keep ROS levels in plant cells low. POD and other antioxidant enzymes assist in converting H_2O_2 to H_2O (Gill and Tuteja, 2010).

The observed findings from SDS-PAGE analysis, as presented in Table 3 and Figure 7, can be elucidated through the understanding that tomato plants possess the capability to adapt to *Fusarium* infection by modulating their biochemical processes. This adaptation causes changes in numerous metabolite activities, which decrease the production of preexisting proteins while simultaneously increasing or creating new proteins that trigger resistance mechanisms (Ullah *et al*., 2018). Prior investigations have elucidated the correlative relationship between the diminishment of bands and the denaturation of enzymes, thereby inducing alterations in the biosynthesis of amino acids and proteins (Dubey and Rani, 1989). The findings presented Figure 7 exhibit concurrence with the observations made by Elbasuney *et al*. (2022), wherein they postulated that the occurrence of biotic stress on plants could potentially elicit either a reduction or augmentation in protein synthesis. The observation of protein bands exhibiting molecular weights of 26, 32, and 37.252 kilodaltons in tomato plants that have undergone infection and subsequent treatment with salicylic acid or fungal endophytes, either independently or in conjunction, can be considered a promising indication for the promotion of systemic resistance, particularly in the context of Chitinase activities.

Conclusions

In the current study, two endophytic *A. tubingensis* and *A. oryzae* were isolated from Healthy *Vigna unguiculata* seeds and identified morphologically and genetically. The encouraging finding of our research suggests that isolated endophytic fungi encourage tomato plants to develop systemic resistance. The endophytic fungal isolates exhibited a notable reduction in the severity of *Fusarium* wilt disease. Tomato plants that treated with *A. tubingensis* and *A. oryzae* and salicylic acid exhibited a notable increase in phenolic compounds, total soluble proteins, total carbohydrates, and photosynthetic pigments. In considering this, endophytic *A. tubingensis* and *A. oryzae* are promising strains for application in agriculture, as an effective biological weapon against *Fusarium* wilt, and for the induction of healthy tomato plants.

Authors' Contributions

Conceptualization: A.M.A. and M.S.A.; Data curation A.M.A., A.H.H and M.S.A; Formal analysis A.M.A., A.H.H and M.S.A; Funding acquisition M.K.A., Y.A.A., H.A.; Investigation A.M.A., A.H.H and M.S.A; Methodology A.M.A. and M.S.A.; Project administration M.S.A; Resources A.M.A., A.H.H and M.S.A; Software A.M.A., A.H.H and M.S.A; Supervision A.M.A., A.H.H and M.S.A; Validation A.M.A., A.H.H and M.S.A; Visualization A.M.A., A.H.H and M.S.A; Writing - original draft A.M.A., A.H.H and M.S.A; Writing - review and editing A.M.A., A.H.H M.K.A., Y.A.A., H.A. and M.S.A. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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