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Biocontrol of Tomato Early Blight Disease Using Some Endophytic Bacterial and Fungal Isolates

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Abstract

Early blight (EB) disease of tomatoes, caused by *Alternaria solani*, is one of the most common and devastating diseases, primarily affecting the plant foliage, stems, and fruits, resulting in severe defoliation, decreased yield, and compromised fruit quality. The current study investigated the potential impact of using endophytic bacterial and fungal isolates for EB disease management and plant growth enhancement. Ten endophytic bacterial and fungal isolates were obtained from the stems and leaves of tomato plants collected from various locations in the Sohag Governorate. These isolates were identified as *Bacillus atrophaeus*, *B. subtilis*, *Pseudomonas corrugata*, *Ps. florsenses*, *Aspergillus nidulans*, *A. terreus*, *Penicillium crustosum*, *Trichoderma hamatum*, *T. harzianum*, and *T. koningii*. During *in vitro* testing, the isolates showed varying levels of antagonistic activity against *A. solani*. All isolates reduced the mycelial radial growth (MRG) of *A. solani*, with *T. harzianum* exhibiting the highest reduction of MRG at 7.4 cm and 82.22% followed by *B. subtilis* and *T. koningii*. In contrast, *P. crustosum* and *Ps. corrugata* showed the lowest reduction of MRG at (1.1 cm and 12.22%) and (1.2 cm and 13.33%), respectively. In the greenhouse and field trails during the 2021 and 2022 growing seasons, the four selected isolates were effective and controlled tomato EB disease, resulting in a decreased percent disease index (PDI) compared to the control. *T. harzianum* and *B. subtilis* were the most effective, showing the highest reduction in PDI and improved plant height, shoot fresh, and dry weight, followed by *T. koningii*. Biochemical analysis of tomato plants after 2, 4, 8, and 16 days of inoculating with *A. solani* revealed that plants inoculated with the selected endophytic bacterial and fungal isolates, especially *T. harzianum* and *B. subtilis*, exhibited the highest increase in total protein content, peroxidase, and polyphenol oxidase activity, and total phenolic content, followed by *T. koningii*. In conclusion, these endophytes have shown potential in managing EB disease, enhancing plant growth, and improving biochemical attributes to combat EB stress in tomato plants.

Keywords: *Alternaria solani*, endophytes, microorganisms, resistance, control.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the world's largest vegetable crops. It is known as a protective food because of its special nutritive value. It provides a major source of minerals and vitamins and is regarded as an anticancer agent (Tan *et al*., 2010). China is the world's top producer of tomatoes (31%), with the United States and India coming in second and third place, respectively (Bais *et al*., 2019). The total area cultivated with tomatoes in Egypt was about 143618 ha in 2022 with a production of 6275443.91 tons and 436954 g/ha of seed yield (FAOSTAT, 2022). The EB disease of tomato is one of the most common and devastating diseases caused by *Alternaria solani* and affects the foliage, stems, and fruits of tomato plants, resulting in severe defoliation, decreased yield, and fruit quality (Chaerani *et al*., 2007). To date, no suitable commercial tomato varieties are available that are resistant to EB disease (Grigolli *et al*., 2011). The management of tomato early blight (EB) has traditionally relied on expensive fungicides, which negatively affect public health and the environment (Hariprasad and Niranjana, 2009). An alternative approach to the management involves the use of antagonistic microorganisms for biological control, which has the potential to effectively manage various root and foliage fungal diseases, including EB (Rabiey *et al.*, 2019; Abdel-Motaal, Fatma *et al.*, 2020a). Many endophytic bacteria and fungi inhabit plant roots, stems, leaves, and fruits without causing harm (Aydi-Ben-Abdallah, Rania *et al.*, 2020; Abdelaziz *et al.*, 2022; Thulasi Bai *et al.*, 2023), and these endophytes offer a promising avenue for controlling plant diseases. Using these endophytic microorganisms as biological control agents has become an attractive, promising, and eco-friendly alternative approach that could inhibit the progress of the target pathogen, limiting disease incidence and severity (de Lamo *et al.*, 2018; Constantin *et al.*, 2019). On many hosts, they act as plant growth-promoting or biocontrol agents by direct antagonism by producing secondary metabolites that can serve as antifungal, antibacterial or via the host by triggering induced resistance as well as growth-promoting agents (Constantin *et al*. 2019; Passari *et al*. 2019). The objectives of this study were to isolate and identify the endophytic microorganisms (Bacteria and fungi) from tomato plants and evaluate their antagonistic activity against the growth of *A. solani in vitro*. Also, their efficacy in controlling EB disease in the greenhouse and field experiments and improving tomato growth was investigated. Another objective was investigating the role of biochemical changes from total protein and phenolic contents and the activation of oxidative enzymes peroxidase and polyphenol oxidase in tomato plants inoculated with these endophytic microorganisms in the physiology of EB disease resistance.

MATERIALS AND METHODS

1. Isolation and identification of the causal pathogen of tomato EB disease:

Isolation was done by directly placing infected leaf portions from diseased tomato samples collected from different localities of Sohag Governorate in 9 cm Petri plates containing potato dextrose agar (PDA) medium supplemented with chloramphenicol (200 mg L^{-1} medium) after disinfecting with 1% sodium hypochlorite (SH) solution for 3 min followed by 4-5 washing with sterile distilled water (SDW) and drying between two sterile filter papers. The plates were then incubated at 27±1 °C for 3-7 days. During incubation, colonies of the growing fungi were checked daily, transferred to PDA plates, and then left for growth at the same conditions. The fungal isolates were purified using single spore and hyphal tip techniques by re-culturing on new PDA plates in the same growth conditions. The pure fungal isolates were identified according to the visual observation of morphological characteristics of the colony, mycelia, and spores described by Domsch *et al.* (1980) using stereo and compound microscopes and then confirmed at the Assiut University Moubasher Mycological Center (AUMMC), Assiut, Egypt. Pure cultures of all fungal isolates were maintained at 5 °C in slants of the PDA medium until use.

2. Pathogenicity tests:

The pathogenicity tests of 11 fungal isolates, 4 belonging to *A. alternata* and 7 to *A. solani,* were conducted in the greenhouse during the 2020 growing season at the Experimental Farm of the Faculty of Agriculture, Sohag University, El-Kawamel, Sohag Governorate. The sowing date was April 15th. The fungal inoculum was prepared by inoculating PDA plates with 5 mm discs from 10-day-old cultures and then incubating the plates at 27±1 °C for 10 days. Subsequently, 10 ml of SDW was added to each plate, and colonies were carefully scraped with a sterile needle to create a conidial suspension, which was then adjusted with SDW to 5×10^5 conidia/ml using a hemocytometer (Awan, Zoia *et al*., 2018). To confirm Koch's postulates, three seeds of the tomato 844 cultivar were sterilized with 1% SH solution for 3 min, washed three times with SDW, left to dry under aseptic conditions, and sown in sterilized plastic pots (25 cm in diameter) filled with sterilized clay-loam soil. Four weeks after the emergence of seedlings, the fungal suspension (20 ml per plant) of each tested isolate was sprayed using an atomizer on tomato leaves (Sallam, Nashwa, 2011) with three replications. Distilled water was used as a control. After inoculating, plants were immediately covered with polyethylene bags to maintain high humidity. After 48 hours, the bags were removed, and the plants were kept under greenhouse conditions and monitored regularly for the development of disease symptoms. Two weeks after inoculation, the disease intensity in each treatment was recorded using a scale of 0-5 described by (Mayee and Datar, 1986). Where 0= No visible symptoms on the leaves, 1= 1-5 percent leaf area infected and covered by spot, no spot on petiole and branches, 2= 6-20 percent leaf area infected and covered by spots, some spots on the petiole, 3= 21-40 percent leaf area infected and covered by spot, spots are also seen on the petiole, branches, 4= 41-70 percent of leaf area infected and covered by spot, spots also seen on the petiole, braches, stem, and 5= >71 percent leaf area infected and covered by spot, spots also seen on the petiole, branch, stem, fruits. The percent disease index (PDI) in each replicate of each tested isolate was calculated using the following formula proposed by Rahmatzai *et al*. (2017).

$$\mathbf{PDI = A/(B \times 5) \times 100}$$

A is the sum of individual disease ratings, B is the number of leaves/ fruits observed, and 5 is the maximum disease grade.

Re-isolations were made from the leaf tissues of the diseased tomato plants under the same conditions as mentioned before, and the cultures thus obtained were compared with the original cultures to confirm the identity and pathogenicity of the EB pathogen.

3. Biological control of tomato EB disease:

3.1. Isolation and identification of endophytic microorganisms from stems and leaves of healthy tomato plants:

Healthy tomato plants observed in the fields infected by EB disease were collected from different localities of the Sohag Governorate, sampled, and used to isolate the endophytic microorganisms. The method described by Duan *et al.* (2013) was followed for isolating the endophytic bacteria from plant samples' stems and leaves. The stems and leaves were cut into small pieces (8 mm). Pieces were rinsed with tap water, dried with absorbent papers, and then immersed in 70% ethanol solution for 30 seconds to remove bubbles on the surface. Then, the pieces were disinfected superficially with 0.1% (w/v) mercuric chloride solution for 2 min and rinsed six times with SDW to eliminate mercuric chloride. The phloem and xylem regions were aseptically separated with a sterile knife and then ground in a sterile mortar with sterile quartz sand and 10 ml of SDW. The phloem and xylem regions were aseptically separated with a sterile knife and then ground in a sterile mortar with sterile quartz sand and 10 ml of SDW. Then, one to ten serial dilutions were made from the well-ground samples using SDW. Then, the target dilution was inoculated in 9 cm Petri dishes containing Nutrient agar (NA) medium. After 24-48 h of incubating at 28 °C, single pure colonies on the NA plates were selected and aseptically transferred to fresh NA medium in slant tubes at 28 °C for 2 days. The bacterial slants were then kept at 4 °C for further studies. The isolated bacteria were identified by the Biolog test using

a Biolog GN microplate (Biolog, Hayward, CA, USA) according to the manufacturer's instructions at the Microorganisms Unit, Plant Pathology Research Institute, Agricultural Research Center, Giza. The endophytic fungi associated with tomato plants were also isolated under aseptic conditions according to the method described by Hazalin *et al.* (2009). Stem and leave samples were respectively washed in tap water, sterilized in 70% ethanol for 1 min followed by 5% SH solution (v/v) for 5 min, and 70% ethanol again for 0.5 min followed by rinsing three times in deionized water, cut into small segments (8 mm), placed in 9 cm Petri dishes containing PDA medium supplemented with streptomycin sulfate 400 mg L^{-1}. The plates were incubated at 28 ±1 °C for 7 days. During incubation, colonies of the growing fungi were checked daily, transferred to new dishes containing PDA medium, and left for growth under the same conditions. The fungal isolates were purified using single spore and hyphal tip techniques by re-culturing on new PDA plates in the same growth conditions. The obtained fungal isolates were identified according to the morphological characteristics of the growing colonies, mycelia, and spores described by Domsch *et al.* (1980 and 2007) and confirmed in the AUMMC, Assiut, Egypt. Pure cultures of all obtained fungal isolates were maintained at 5 °C in slants of the PDA medium until use.

3.2. Antagonistic activity of isolated endophytic microorganisms against *A. solani in vitro*:

Ten isolated endophytic microorganisms (4 bacterial and 6 fungal isolates) listed in Table 3 from stems and leaves of healthy tomato plants were tested to investigate their antagonistic activity against *A. solani in vitro*. Sterilized Petri dishes (9 cm) containing PDA medium were inoculated with discs (5 mm) of *A. solani* from the 7-day-old culture in the dishes center. At two opposite peripheries of the dish, two discs (5 mm) were also inoculated with each tested endophytic fungal isolate. On the other hand, the Petri dishes' other side was inoculated with *A. solani* discs, and a streak of each endophytic bacterial isolate was done close to the dishes' periphery. Control dishes were only inoculated with discs of *A. solani* in the dishes center. Four dishes were used as replicates for each treatment in a completely randomized design. Inoculated dishes were then incubated at 28 °C till the control plates were wholly covered with mycelium. The experiment was repeated twice, and the mycelial linear growth reduction of *A. solani* as the diameter (cm) was measured. The growth reduction (%) was then calculated for each replicate of each tested endophytic isolate according to the equation as follows:

Growth reduction (%) = Growth in control – growth in treatment/ growth in control × 100

Data were represented as the means of the two conducted experiments.

3.3. Effect of selected bacterial and fungal isolates on controlling tomato EB disease:

3.3.1. Greenhouse experiments:

Pot experiments were conducted under greenhouse conditions at the Experimental Farm, Faculty of Agriculture, Sohag University, El-Kawamel, Sohag, during the 2021 and 2022 growing seasons to evaluate some tomato cultivars' susceptibility to infection by *A. solani*. The sowing date in both experiments was the 15th of April. Inoculum of *A. solani* was prepared by inoculating PDA plates with 5 mm discs from the 10-day-old culture and then incubating plates at 27±1 °C for 10 days. Then, 10 ml of SDW was added to each plate, and colonies were carefully scraped with a sterile needle to make a conidial suspension, adjusted to a concentration of 5 × 10^5 conidia/ml, as mentioned before. Inocula of 10 microbial endophytes *B. subtilis*, *Ps. florsenses*, *T. harzianum,* and *T. koningii* were prepared by growing each microbial endophyte in 300 ml flasks containing 100 ml NA broth medium for bacteria and PDA broth medium for fungi and shaking using a horizontal rotary shaker (150 rpm) at 28 °C for 2 days for bacteria and 7 days for fungi. Seeds of tomato cultivar 844 were surface sterilized by immersion in 2% SH solution for 2 min, then rinsed in SDW. The disinfected seeds were soaked in the inocula of the tested microbial endophytes for 2 h and then air-dried under aseptic conditions before being planted in sterilized 30 cm plastic pots filled

with sterilized clay-loam soil at the rate of 3 seeds per pot and 8 pots (replicates) were used for each treatment in a completely randomized block experimental design then pots were slightly irrigated every other day, as mentioned before. Four weeks after the emergence of seedlings, the leaves were inoculated with *A. solani* using an atomizer (20 ml/plant), as mentioned before. After inoculation, plants were immediately covered with polyethylene bags to maintain high humidity conditions. After 48 hours, the bags were removed, and the plants were kept under greenhouse conditions and monitored regularly for the development of EB disease symptoms. Two weeks after inoculation, the disease intensity was recorded using a scale of 0-5, and the PDI was then calculated, as mentioned before. Data of the means over the two growing seasons, 2021 and 2022, were also calculated and analyzed.

3.3.2. Field experiments:

Under field conditions and artificial infestation with *A. solani*, the following experiments were conducted in the Experimental Farm, Faculty of Agriculture, Sohag University, El-Kawamel, Sohag University, during the 2021 and 2022 growing seasons to evaluate the biocontrol efficiency of 4 endophytic bacterial and fungal isolates as bioagents against *A. solani* causing EB disease of tomato. The sowing date in both experiments was the 15th of April. Inoculum of *A solani* was prepared by inoculating PDA plates with 5 mm discs from the 10-day-old culture and then incubating plates at 27±1 °C for 10 days. Then, 10 ml of SDW was added to each plate, and colonies were carefully scraped with a sterile needle to make a conidial suspension, adjusted to a concentration of 5 × 10^5 conidia/ml, as mentioned before. Also, inocula of selected microbial endophytes *B. subtilis*, *Ps. florsenses*, *T. harzianum*, and *T. koningii* were prepared by growing each microbial endophyte in 300 ml flasks containing 100 ml NA broth medium for bacteria and PDA broth medium for fungi on a horizontal rotary shaker (150 rpm) at 28 °C for 2 days for bacteria and 7 days for fungi as mentioned before. Seeds of tomato cultivar 844 were surface sterilized by immersion in 2% SH solution for 2 min, then rinsed in SDW. The disinfected seeds were soaked in the inocula of the tested microbial endophytes for 2 h and then air-dried under aseptic conditions before being planted in the soil. In each experiment, the inoculated seeds were planted in hills on rows of plots in a randomized complete block experimental design. Each row was 3.0 m long, with 0.6 m and 20 cm between hills within rows, and 12 hills were per row. Three rows represented each tested isolate of microbial endophytes as replications in each plot. All cultural practices recommended for bean production were followed. Four weeks after the emergence of seedlings, the leaves were inoculated with *A. solani* using an atomizer (20 ml/plant), as mentioned before. The tomato plants were then monitored regularly for EB symptoms development. Two weeks after inoculation, the disease intensity was recorded using a scale of 0-5, and the PDI was then calculated, as mentioned before. Data of the means over the two growing seasons, 2021 and 2022, were also calculated and analyzed.

3.4. Effect of selected endophytic bacterial and fungal isolates on plant vegetative growth of tomato under field conditions:

Following the conducted field trials in the 2021 and 2022 growing seasons mentioned above, ten tomato plant samples of each treatment were randomly selected after 70 days of planting to assess plant vegetative growth (PVG) parameters of plant height (cm), shoot fresh and dry weight (g) with all aerial part and the means were then calculated. Data of the means over the two growing seasons, 2021 and 2022, were also calculated and analyzed.

3.5. Biochemical changes of tomato plants inoculated with selected endophytic bacterial and fungal isolates in response to the infection by *A. solani* causing EB disease:

Following the conducted field trials in the 2021 and 2022 growing seasons mentioned before, three tomato plant samples of each treatment were randomly selected to investigate the biochemical changes of tomato plants 2, 4, 8, and 16 after inoculation with *A. solani*. This

study aimed to determine the total protein content, peroxidase (PO) activity, polyphenol oxidase (PPO) activity, and phenolic content in inoculated tomato plants with the endophytic bacterial and fungal isolates in response to the infection by *A. solani*.

3.5.1. Total protein content:

The total protein contents of the whole plant samples of each tested bioagent endophyte trent were determined according to the method described by Bradford (1976) using crystalline bovine serum albumin (BSA) as a standard. The stems and leaves plant samples from each endophyte treatment were collected 2, 4, 8, and 16 days after inoculation with *A. solani*, and then 1 g of each plant was heated at 85 °C with 1 N NaOH. The hydrolyzed protein was then determined using Bio-Rad assay dye, and the developed color was measured at 595 nm. The total protein content in each tested sample was calculated as mg g^{-1} fresh weight from the standard curve of BSA. Data were then represented as the means over the two growing seasons, 2021 and 2022.

3.5.2. Activity of oxidative enzymes PO and PPO:

The PO and PPO enzyme extraction was performed according to the method described by Maxwell and Bateman (1967). Stems and leaves tissue samples (1 g fresh weight) were ground in a sterile mortar with 10 ml of 0.1 M phosphate buffer (pH= 7) and strained through layers of sterile muslin cloths. Each sample's extract of stem and leaf tissues was filtrated by centrifuging for 10 min at 2500 g and 4 °C, and the supernatant was then used as enzyme extract. A reaction mixture contained 0.5 ml of freshly dissolved 0.5% Catechol, 1 ml of 0.1 M phosphate buffer, 4.5 ml SDW, and 0.2 ml of enzyme extract. The activity of PO and PPO enzymes was determined by measuring the absorbance at 470 and 480 nm for PO and PPO, respectively, after 15 min. Then, the PO and PPO activity was expressed as absorbance g^{-1} fresh weight 15 min^{-1}. Data were represented as the means over the two growing seasons, 2021 and 2022.

3.5.3. Total phenolic content:

Fresh stems and leaves (1 g) of each tested sample of each microbial endophyte treatment were ground and extracted in 50 % methanol (12 v:v) for 90 min at 80 °C to extract and determine the total phenolic contents. The extract was centrifuged at 14000 g for 15 min, and then the supernatant was used to determine free and cell wall-bound phenolics using the Folin–Ciocaleus (FC) reagent according to the method described by Kofalvi and Nassuth (1995). The pellet was saponified with 2 ml of 0.5 N NaOH for 24 h at room temperature to release the bound phenolics, neutralized with 0.5 ml 2 N HCl, and then centrifuged at 14000 g for 15 min. The supernatant was used to bind phenolic determination using an FC assay. Extracts of the methanol and NaOH (100 µL) were diluted to 1.0 ml with distilled water and mixed with 0.5 ml of 2 N FC reagent and 2.5 ml of 20% Na_2 CO_3. The mixture was allowed to stand in the dark for 20 min at room temperature, and the absorbance of samples was then measured at 725 nm by spectrophotometer. A stock solution (1 mg ml^{-1}) of gallic acid was prepared in distilled water. Then, various concentrations ranging from 1 to 10 µg ml^{-1} were prepared. To each used concentration, 1.5 ml of FC reagent was added and kept for 5 min, and then 4 ml of 20% Na_2 CO_3 solution was added and completed up to 10 ml with distilled water. Then, the mixture was kept for 20 min, and absorbance was measured at 725 nm. The samples' total phenolic concentration (µg ml^{-1}) was extrapolated from a standard curve constructed using gallic acid as a standard. The absorbance values were then converted to mg of total phenolics g^{-1} of fresh weight. Data were represented as the means over the two growing seasons, 2021 and 2022.

Statistical analysis:

This study's data were statistically analyzed using the MSTAT-C program version 2.10. Duncan's multiple range tests for means comparing and the least significant difference (L.S.D.) at the *P*= 0.05 probability level was used as described by Gomez and Gomez (1984).

RESULT

1. Isolation and identification of the causal pathogen of tomato EB disease:

Table 1 shows that eleven purified fungal isolates belonging to the genus *Alternaria* were obtained from diseased tomato plants of different grown cultivars showing EB symptoms collected from different localities in Sohag Governorate. All fungal isolates were identified based on their morphological characteristics of the colony, mycelia, and spores as *Alternaria alternata* (Fr.) Keissl. (4 isolates) and *Alternaria solani* Sorauer (7 isolates).

2. Pathogenicity tests:

The pathogenic capabilities of 11 fungal isolates of *A. alternata* (4 isolates) and *A. solani* (7 isolates) were studied on the tomato cultivar 025 to induce EB symptoms under greenhouse conditions during the 2020 growing season. Results in Table 2 and Figure 1 a and b show that the tested fungal isolates of *A. solani* were highly pathogenic to tomato plants, causing typical EB disease symptoms. However, these isolates significantly differed in their virulence on tomato plants to cause EB disease. In this regard, isolates No. 11 and 6 caused the highest PDI (92 and 90.5%), followed by isolate No. 7 with 86.54% of the PDI of tomato EB. In contrast, isolate No. 5 caused the lowest PDI (49.5%) of EB. The isolates No. 5, 8, and 10 caused PDI values ranging from 57.83 to 75.40% of EB. On the other hand, the tested four isolates of *A. alternata* had weak virulence on tomato plants, causing light blight symptoms with considerable PDI values ranging from 25.08 to 29.99%. Based on the data obtained, the seven isolates of *A. solani* could be characterized as highly virulent (isolates No. 11 and 6), moderately virulent (isolates No. 5, 8, and 10), and low virulent (isolate No. 5). Re-isolation from infected tomato plants showed typical *A. solani* isolates similar to the original ones.

3. Biological control of EB disease:

3.1. Isolation and identification of endophytic microorganisms from stems and leaves of healthy tomato plants:

Data in Table 3 shows that 10 endophytic microorganisms, including 4 bacterial and 6 fungal isolates, were isolated from stems and leaves of tomato plants collected from different localities in the Sohag Governorate. Based on the Biolog test, the obtained endophytic bacterial isolates were identified as *Bacillus atrophaeus* Nakamura, *Bacillus subtilis* Cohn, *Pseudomonas corrugata* Roberts and Scarlett, and *Pseudomonas florsenses* Migula. Also, the endophytic fungal isolates were identified based on the morphological characteristics of the growing colonies, mycelia, and spores to be *Aspergillus nidulans* G. Winter, *Aspergillus terreus* Thom, *Penicillium crustosum* Thom, *Trichoderma hamatum* (Bonord.) Bainier, *Trichoderma harzianum* Rifai, and *Trichoderma koningii* Oudeman H. Barakats.

3.2. Antagonistic activity of isolated endophytic microorganisms against *A. solani in vitro*:

Ten endophytic 4 bacterial and 6 fungal isolates isolated from stems and leaves of healthy tomato plants, were tested to investigate their antagonistic activity against *A. solani in vitro*. Results in Table 4 and Fig. 2 showed that all the tested endophytic bacterial and fungal isolates varied significantly in their antagonistic activity against *A. solani*. All the tested fungal and bacterial isolates reduced the mycelial radial growth of *A. solani*. *T. harzianum* was the most antagonistic microorganism and caused the highest reduction of mycelial radial growth with 7.4 cm and 82.22% of *A. solani*, followed by *B. subtilis* and *T. koningii*, where they caused a reduction of mycelial radial growth with (5.4 cm and 60%) and (5.9 cm and 65.56%), respectively. In contrast, *Pe. crustosum* and *Ps. corrugata* caused the lowest reduction of the mycelial radial growth, with (1.1 cm and 12.22%) and (1.2 cm and 13.33%), respectively. On the other hand, the rest of the isolated endophytic bacterial and fungal isolates caused a

reduction of mycelial radial growth ranging from 1.9 cm and 21.11% to 2.6 cm and 28.89%.

3.3. Effect of endophytic bacterial and fungal isolates on controlling tomato EB disease:

3.3.1. Greenhouse experiments:

Under greenhouse conditions in the 2021 and 2022 growing seasons, the selected four endophytic bacterial and fungal isolates were tested for their effects on the incidence of tomato EB disease caused by *A. solani*. Results in Table 5 show that the tested endophytic bacterial and fungal isolates varied significantly in controlling tomato EB disease, where they decreased the PDI in both growing seasons 2021 and 2022 compared to the control of non-treated plants. *T. harzianum* and *B. subtilis* were the most effective endophytic microorganisms exhibiting the highest reduction in the PDI of tomato EB in 2021 and 2022, reaching (25.56 and 25.67%) with a mean of 25.62% and (31.67 and 31.86%) with a mean of 31.77%, followed by *T. koningii* (35.90 and 35.83%) in 2021 and 2022, respectively with a mean of 35.87%. In contrast, *Ps. florsenses* was the less effective endophytic microorganism and exhibited the lowest reduction in the PDI of EB, reaching 45.83 and 45.90% in 2021 and 2022, respectively, with a mean of 45.87%.

3.3.2. Field experiments:

In the growing seasons 2021 and 2022 under field conditions, the selected four endophytic bacterial and fungal isolates were also tested for their effects on the incidence of tomato EB disease caused by *A. solani*. Results in Table 6 show that the tested endophytic bacterial and fungal isolates varied significantly in controlling tomato EB disease, where they decreased the PDI in both seasons 2021 and 2022 compared to the control of non-treated plants. *T. harzianum* and *B. subtilis* were the most effective endophytic microorganisms, exhibiting the highest reduction in the PDI of tomato EB in 2021 and 2022, reaching (34.83 and 34.67%) with a mean of 34.75% and (37.67 and 36.83%) with a mean of 37.25%, followed by *T. koningii* (42.33 and 41.83%) in 2021 and 2022, respectively with a mean of 42.08%. In contrast, *Ps. florsenses* was the less effective endophytic microorganism and exhibited the lowest reduction in the PDI of EB, reaching 47.67 and 46.41% in 2021 and 2022, respectively, with a mean of 47.04%.

3.3.3. Effect of endophytic bacterial and fungal isolates on plant vegetative growth of tomato under field conditions:

In the growing seasons 2021 and 2022 under field conditions, the selected four endophytic bacterial and fungal isolates were also tested for their effects on some plant vegetative growth parameters of plant height (cm), shoot fresh and dry weight (g) with all aerial part in response to infection by *A. solani* causing EB disease. Results in Table 7 show that the tested endophytic bacterial and fungal isolates varied significantly in their affecting plant vegetative growth parameters, where they increased the plant height and shoot fresh and dry weight in both seasons 2021 and 2022 compared to the control of non-treated plants. *T. harzianum* and *B. subtilis* were the most effective endophytic microorganisms, exhibiting the highest increase in plant height and the shoot fresh and dry weight, reaching (39.69 cm, 243.75 g, and 22.75 g) and (39.47 cm, 241.65 g, and 22.35 g) in 2021 and 2022 with a mean of 39.58 cm, 242.70 g, and 22.55 g and (38.35 cm, 237.85 g, and 21.55 g) and (38.24 cm, 236.63 g, and 21.09 g) with a mean of 38.29 cm, 237.24 g, and 21.32 g, followed by *T. koningii* (37.51 cm, 231.69 g, and 20.36 g) and (37.39 cm, 230.74 g, and 20.05 g) in 2021 and 2022 with a mean of 37.45 cm, 231.22 g, and 20.21 g. In contrast, *Ps. florsenses* was the less effective endophytic microorganism and exhibited the lowest increase in the plant height and shoot fresh and dry weight in 2021 and 2022, reaching (36.39 cm, 225.71 g, and 18.45 g) and (36.11 cm, 223.45 g, and 17.89 g), respectively with a mean of 36.25 cm, 224.58 g, and 18.17 g. endophytic bacterial and

3.4. Biochemical changes of tomato plants inoculated with selected fungal isolates in response to the infection by *A. solani* causing EB disease:

3.4.1. Total protein content:

Table 8 shows the total protein contents of tomato plants inoculated with 4 endophytic

bacterial and fungal isolates after 2, 4, 8, and 16 days of inoculating with *A. solani* under field conditions in the 2021 and 2022 growing seasons. Total protein content of tomato plants non-inoculated with endophytic bacterial and fungal isolates was estimated after 0, 2, 4, 8, and 16 days of inoculating with *A. solani* (control) as 48±0.81, 55±1.63, 59±0.81, 67±1.63, and 79±0.81 mg g^{-1} fresh weight, respectively with a mean of 61.6±1.14 mg g^{-1} fresh weight. At the same time, these amounts gradually increased after 2, 4, 8, and 16 days of inoculating with *A. solani* in plants inoculated with the endophytic bacterial and fungal isolates. Results also show that *T. harzianum* and *B. subtilis* were the most effective endophytic microorganisms, inducing the highest increase in total protein contents after 0, 2, 4, 8, and 16 days of tomato plants inoculated with *A. solani*, reaching (58±1.63, 64±1.63, 70±1.63, 81±1.63 and 94±1.63 mg g^{-1} fresh weight, respectively) with a mean of 73.4±1.63 mg g^{-1} fresh weight and (56±1.63, 61±1.63, 66±0.81, 77±1.63 and 90±1.63 mg g^{-1} fresh weight, respectively) with a mean of 70±1.47 mg g^{-1} fresh weight, followed by *T. koningii* (53±1.63, 59±1.63, 63±1.63, 74±1.63 and 86±1.63 mg g^{-1} fresh weight, respectively) with a mean of 67±1.63 mg g^{-1} fresh weight compared to the control of non-inoculated plants. In contrast, *Ps. florsenses* was the less effective endophytic microorganism and induced the lowest increase in the total protein contents, reaching (50±0.81, 57±1.63, 60±0.81, 71±0.81 and 81±0.81 mg g^{-1} fresh weight, respectively) with a mean of 63.8±0.97 mg g^{-1} fresh weight.

3.4.2. PO activity:

Table 9 shows the PO activity of tomato plants inoculated with 4 endophytic bacterial and fungal isolates after 2, 4, 8, and 16 days of inoculating with *A. solani* under field conditions in the 2021 and 2022 growing seasons. The PO activity of tomato plants non-inoculated with endophytic bacterial and fungal isolates was estimated after 0, 2, 4, 8, and 16 days of inoculating with *A. solani* (control) as 0.196±0.001, 0.207±0.003, 0.219±0.001, 0.237±0.003, and 0.256±0.001 absorbance g^{-1} fresh weight 15 min^{-1}, respectively with a mean of 0.223±0.002 absorbance g^{-1} fresh weight 15 min^{-1}. At the same time, these amounts gradually increased after 2, 4, 8, and 16 days of inoculating with *A. solani* in plants inoculated with the endophytic bacterial and fungal isolates. Results also show that *T. harzianum* and *B. subtilis* were the most effective endophytic microorganisms, inducing the highest increase in the PO activity after 0, 2, 4, 8, and 16 days of tomato plants inoculated with *A. solani*, reaching (0.206±0.003, 0.244±0.001, 0.261±0.001, 0.281±0.002 and 0.317±0.002 absorbance g^{-1} fresh weight 15 min^{-1}, respectively) with a mean of 0.262±0.002 absorbance g^{-1} fresh weight 15 min^{-1} and (0.203±0.003, 0.235±0.001, 0.243±0.003, 0.269±0.001 and 0.294±0.003 absorbance g^{-1} fresh weight 15 min^{-1}, respectively) with a mean of 0.249±0.002 absorbance g^{-1} fresh weight 15 min^{-1}, followed by *T. koningii* (0.201±0.003, 0.227±0.003, 0.235±0.002, 0.253±0.001 and 0.277±0.003 absorbance g^{-1} fresh weight 15 min^{-1}, respectively) with a mean of 0.239±0.002 absorbance g^{-1} fresh weight 15 min^{-1} compared to the control plants. In contrast, *Ps. florsenses* was the less effective endophytic microorganism and induced the lowest increase in the PO activity, reaching (0.199±0.002, 0.219±0.001, 0.226±0.003, 0.241±0.001 and 0.263±0.003 absorbance g^{-1} fresh weight 15 min^{-1}, respectively) with a mean of 0.229±0.002 absorbance g^{-1} fresh weight 15 min^{-1}.

3.4.3. PPO activity:

Table 10 shows the PPO activity of tomato plants inoculated with 4 endophytic bacterial and fungal isolates after 2, 4, 8, and 16 days of inoculating with *A. solani* under field conditions in the 2021 and 2022 growing seasons. The PPO activity of tomato plants non-inoculated with endophytic bacterial and fungal isolates was estimated after 0, 2, 4, 8, and 16 days of inoculating with *A. solani* (control) as 0.093±0.002, 0.101±0.003, 0.111±0.002, 0.117±0.003, and 0.121±0.002 absorbance g^{-1} fresh weight 15 min^{-1}, respectively with a mean of 0.109±0.002 absorbance g^{-1} fresh weight 15

min^{-1}. At the same time, these amounts gradually increased after 2, 4, 8, and 16 days of inoculating with *A. solani* in plants inoculated with the endophytic bacterial and fungal isolates. Results also show that *T. harzianum* and *B. subtilis* were the most effective endophytic microorganisms, inducing the highest increase in the PPO activity after 0, 2, 4, 8, and 16 days of tomato plants inoculated with *A. solani*, reaching (0.103±0.001, 0.114±0.003, 0.121±0.003, 0.133±0.001 and 0.144±0.002 absorbance g^{-1} fresh weight 15 min^{-1}, respectively) with a mean of 0.123±0.002 absorbance g^{-1} fresh weight 15 min^{-1} and (0.101±0.002, 0.109±0.003, 0.117±0.003, 0.125±0.001 and 0.134±0.003 absorbance g^{-1} fresh weight 15 min^{-1}, respectively) with a mean of 0.117±0.002 absorbance g^{-1} fresh weight 15 min^{-1}, followed by *T. koningii* (0.098±0.002, 0.106±0.003, 0.115±0.002, 0.122±0.001 and 0.127±0.003 absorbance g^{-1} fresh weight 15 min^{-1}, respectively) with a mean of 0.114±0.002 absorbance g^{-1} fresh weight 15 min^{-1} compared to the control plants. In contrast, *Ps. florsenses* was the less effective endophytic microorganism and induced the lowest increase in the PPO activity, reaching (0.095±0.003, 0.103±0.002, 0.113±0.003, 0.119±0.002 and 0.125±0.003 absorbance g^{-1} fresh weight 15 min^{-1}, respectively) with a mean of 0.111±0.003 absorbance g^{-1} fresh weight 15 min^{-1}.

3.4.4. Total phenolic content:

Table 11 shows the total phenolic content of tomato plants inoculated with 4 endophytic bacterial and fungal isolates after 2, 4, 8, and 16 days of inoculating with *A. solani* under field conditions in the 2021 and 2022 growing seasons. The total phenolic content of tomato plants non-inoculated with endophytic bacterial and fungal isolates was estimated after 0, 2, 4, 8, and 16 days of inoculating with *A. solani* (control) as 1.412±0.012, 1.422±0.013, 1.347±0.012, 1.364±0.013, and 1.385±0.012 mg g^{-1} of fresh weight, respectively with a mean of 1.351±0.012 mg g^{-1} of fresh weight. At the same time, these amounts gradually increased after 2, 4, 8, and 16 days of inoculating with *A. solani* in plants inoculated with the endophytic bacterial and fungal isolates. Results also showed that *T. harzianum* and *B. subtilis* were the most effective endophytic microorganisms, inducing the highest increase in the total phenolic content after 0, 2, 4, 8, and 16 days of tomato plants inoculated with *A. solani*, reaching (1.441±0.011, 1.453±0.013, 1.466±0.013, 1.478±0.011 and 1.489±0.012 mg g^{-1} of fresh weight, respectively) with a mean of 1.465±0.012 mg g^{-1} of fresh weight and (1.438±0.012, 1.445±0.013, 1.458±0.013, 1.467±0.011 and 1.478±0.013 mg g^{-1} of fresh weight, respectively) with a mean of 1.457±0.013 mg g^{-1} of fresh weight, followed by *T. koningii* (1.431±0.012, 1.439±0.013, 1.449±0.012, 1.461±0.011 and 1.471±0.013 mg g^{-1} of fresh weight, respectively) with a mean of 1.450±0.012 mg g^{-1} of fresh weight compared to the control plants. In contrast, *Ps. florsenses* was the less effective endophytic microorganism and induced the lowest increase in the total phenolic content, reaching (1.429±0.011, 1.435±0.012, 1.441±0.013, 1.451±0.012 and 1.462±0.013 mg g^{-1} of fresh weight, respectively) with a mean of 1.443±0.012 mg g^{-1} of fresh weight.

Table 1: Source and identification of 11 fungal isolates obtained from diseased tomato plants showing EB symptoms collected from different localities in Sohag Governorate.

Fungal isolate			Identification
No.	Source		
	Locality	Cultivar	
1	El Monshah	588	*Alternaria alternata* (Fr.) Keissl.
2	Girga	844	
3	Sohag	Castle Rock	
4	Baliana	Super gold	
5	El Monshah	588	*Alternaria solani* Sorauer
6	Girga	844	
7	Sohag	844	
8	Baliana	Super gold	
9	Tema	Castle Rock	
10	Johenna	Castle Rock	
11	El Maragha	844	

Table 2: Pathogenic capabilities of 11 fungal isolates of *A. alternata* (4 isolates) and *A. solani* (7 isolates) on tomato cv. 844 to cause EB disease under greenhouse conditions during the 2020 growing season.

Fungal isolate No.	PDI
A. alternata	
1	25.08
2	29.99
3	27.17
4	26.50
A. solani	
5	57.83
6	90.50
7	86.54
8	76.00
9	49.50
10	75.40
11	92.00
General control*	0.00
Mean	**57.86**
L.S.D. at 0.05	5.21

* Tomato plants sprayed with sterilized water.

Table 3: Source and identification of 10 endophytic bacterial and fungal isolates isolated from healthy tomato plants.

Isolate No.	Source		Identification
	Part	Locality	
1	Leaves	Tema	*Bacillus atrophaeus* Nakamura
2	Leaves	Tema	*Bacillius subtilis* Cohn
3	Leaves	Baliana	*Pseudomonas corrugata* Roberts and Scarlett
4	Leaves	El Monshah	*Pseudomonas florsenses* Migula
5	Stem	Tahta	*Aspergillus nidulans* G. Winter
6	Stem	El Monshah	*Aspergillus terreus* Thom
7	Stem	Girga	*Penicillium crustosum* Thom
8	Stem	Johenna	*Trichoderma hamatum* (Bonord.) Bainier
9	Stem	El Maragha	*Trichoderma harzianum* Rifai
10	Stem	Girga	*Trichoderma koningii* Oudeman H. Barakats

Table 4: Antagonistic activity of 10 endophytic bacterial and fungal isolates against *A. solani in vitro*.

Endophytic isolate	Growth reduction	
	Diameter (cm)	(%)
B. atrophaeus	2.2	24.44
B. subtilis	5.4	60.00
Ps. corrugata	1.2	13.33
Ps. florsenses	2.6	28.89
A. nidulans	1.4	15.56
A. terreus	1.6	17.78
Pe. crustosum	1.1	12.22
T. hamatum	1.9	21.11
T. harzianum	7.4	82.22
T. koningii	5.9	65.56
Control	0.0	0.00
Mean	**2.7**	**31.01**
L.S.D. at 0.05	0.4	6.51

Table 5: Effect of endophytic bacterial and fungal isolates on controlling tomato EB disease caused by *A. solani* in the 2021 and 2022 growing seasons under greenhouse conditions.

Endophytic isolate	PDI		
	2021	2022	Mean
B. subtilis	31.67	31.86	31.77
Ps. florsenses	45.83	45.90	45.87
T. harzianum	25.56	25.67	25.62
T. koningii	35.90	35.83	35.87
Control	90.67	89.17	89.92
Mean	45.93	45.69	45.81
L.S.D. at 0.05	4.71	4.49	4.67

Table 6: Effect of endophytic bacterial and fungal isolates on controlling tomato EB disease caused by *A. solani* in the 2021 and 2022 growing seasons under field conditions.

Endophytic isolate	PDI		
	2021	2022	Mean
B. subtilis	37.67	36.83	37.25
Ps. florsenses	47.67	46.41	47.04
T. harzianum	34.83	34.67	34.75
T. koningii	42.33	41.83	42.08
Control	88.83	87.41	89.92
Mean	50.63	49.78	50.21
L.S.D. at 0.05	2.95	2.98	2.92

Table 7: Effect of endophytic bacterial and fungal isolates on plant vegetative growth (PVG) parameters of tomato in response to infection by *A. solani* causing EB disease in the 2021 and 2022 growing seasons under field conditions.

Endophytic isolate	PVG parameters								
	2021			2022			Mean		
	Plant height (cm)	Shoot		Plant height (cm)	Shoot		Plant height (cm)	Shoot	
		Fresh weight (g)	Dry weight (g)		Fresh weight (g)	Dry weight (g)		Fresh weight (g)	Dry weight (g)
B. subtilis	38.35	237.85	21.55	38.24	236.63	21.09	**38.29**	**237.24**	**21.32**
Ps. florsenses	36.39	225.71	18.45	36.11	223.45	17.89	**36.25**	**224.58**	**18.17**
T. harzianum	39.69	243.75	22.75	39.47	241.65	22.35	**39.58**	**242.70**	**22.55**
T. koningii	37.51	231.69	20.36	37.39	230.74	20.05	**37.45**	**231.22**	**20.21**
Control	30.24	209.87	16.87	29.75	208.87	16.39	**29.99**	**209.37**	**16.63**
Mean	**36.44**	**229.75**	**19.99**	**36.19**	**228.27**	**19.56**	**36.31**	**229.01**	**19.78**
L.S.D. at 0.05	1.21	3.23	0.73	1.20	3.21	0.71	1.20	3.22	0.72

Table 8: Effect of endophytic bacterial and fungal isolates on the total protein content of tomato plants after 0, 2, 4, 8, and 16 days of inoculating with *A. solani* under field conditions in the 2021 and 2022 growing seasons.

Endophytic isolate	Total protein content (mg g^{-1} fresh weight)*					
	0 days***	2 days	4 days	8 days	16 days	Mean
B. subtilis	56±1.63****	61±1.63	66±0.81	77±1.63	90±1.63	**70.0±1.47**
Ps. florsenses	50±0.81	57±1.63	60±0.81	71±0.81	81±0.81	**63.8±0.97**
T. harzianum	58±1.63	64±1.63	70±1.63	81±1.63	94±1.63	**73.4±1.63**
T. koningii	53±1.63	59±1.63	63±1.63	74±1.63	86±1.63	**67.0±1.63**
Control**	48±0.81	55±1.63	59±0.81	67±1.63	79±0.81	**61.6±1.14**
Mean	**53±1.30**	**59.2±1.63**	**63.6±1.14**	**74±1.47**	**86±1.30**	**67.2±1.37**

* Data are the means over the two growing seasons, 2021 and 2022.
** Plants inoculated with *A. solani*.
*** Before inoculating plants with *A. solani*.
**** Values are the means (mg g^{-1} fresh weight ± standard deviation) over three replicates from the standard curve of BSA.

Table 9: Effect of endophytic bacterial and fungal isolates on the PO activity of tomato plants after 0, 2, 4, 8, and 16 days of inoculating with *A. solani* under field conditions in the 2021 and 2022 growing seasons.

Endophytic isolate	PO activity*					
	0 days***	2 days	4 days	8 days	16 days	Mean
B. subtilis	0.203±0.003****	0.235±0.001	0.243±0.003	0.269±0.001	0.294±0.003	**0.249±0.002**
Ps. florsenses	0.199±0.002	0.219±0.001	0.226±0.003	0.241±0.001	0.263±0.003	**0.229±0.002**
T. harzianum	0.206±0.003	0.244±0.001	0.261±0.001	0.281±0.002	0.317±0.002	**0.262±0.002**
T. koningii	0.201±0.003	0.227±0.003	0.235±0.002	0.253±0.001	0.277±0.003	**0.239±0.002**
Control**	0.196±0.001	0.207±0.003	0.219±0.001	0.237±0.003	0.256±0.001	**0.223±0.002**
Mean	**0.201±0.002**	**0.226±0.002**	**0.237±0.002**	**0.256±0.002**	**0.281±0.002**	**0.240±0.002**

* Data are the means over the two growing seasons, 2021 and 2022.
** Plants inoculated with *A. solani*.
*** Before inoculating plants with *A. solani*.
**** Values are the means (absorbance ± standard deviation g^{-1} fresh weight 15 min^{-1}) over three replicates.

Table 10: Effect of endophytic bacterial and fungal isolates on the PPO activity of tomato plants after 0, 2, 4, 8, and 16 days of inoculating with *A. solani* under field conditions in the 2021 and 2022 growing seasons.

Endophytic isolate	PPO activity*					
	0 days***	2 days	4 days	8 days	16 days	Mean
B. subtilis	0.101±0.002****	0.109±0.003	0.117±0.003	0.125±0.001	0.134±0.003	**0.117±0.002**
Ps. florsenses	0.095±0.003	0.103±0.002	0.113±0.003	0.119±0.002	0.125±0.003	**0.111±0.003**
T. harzianum	0.103±0.001	0.114±0.003	0.121±0.003	0.133±0.001	0.144±0.002	**0.123±0.002**
T. koningii	0.098±0.002	0.106±0.003	0.115±0.002	0.122±0.001	0.127±0.003	**0.114±0.002**
Control**	0.093±0.002	0.101±0.003	0.111±0.002	0.117±0.003	0.121±0.002	**0.109±0.002**
Mean	**0.098±0.002**	**0.107±0.003**	**0.115±0.001**	**0.123±0.002**	**0.130±0.003**	**0.115±0.002**

* Data are the means over the two growing seasons, 2021 and 2022.
** Plants inoculated with *A. solani*.
*** Before inoculating plants with *A. solani*.
**** Values are the means (absorbance ± standard deviation g^{-1} fresh weight 15 min^{-1}) over three replicates.

Table 11: Effect of endophytic bacterial and fungal isolates on the total phenolic content of tomato plants after 0, 2, 4, 8, and 16 days of inoculating with *A. solani* under field conditions in the 2021 and 2022 growing seasons.

Endophytic isolate	Total phenolic contents (mg of phenolics g^{-1} of fresh weight)*					
	0 days***	2 days	4 days	8 days	16 days	Mean
B. subtilis	1.438±0.012****	1.445±0.013	1.458±0.013	1.467±0.011	1.478±0.013	**1.457±0.013**
Ps. florsenses	1.429±0.011	1.435±0.012	1.441±0.013	1.451±0.012	1.462±0.013	**1.443±0.012**
T. harzianum	1.441±0.011	1.453±0.013	1.466±0.013	1.478±0.011	1.489±0.012	**1.465±0.012**
T. koningii	1.431±0.012	1.439±0.013	1.449±0.012	1.461±0.011	1.471±0.013	**1.450±0.012**
Control**	1.321±0.012	1.339±0.013	1.347±0.012	1.364±0.013	1.385±0.012	**1.351±0.012**
Mean	**1.412±0.012**	**1.422±0.013**	**1.432±0.013**	**1.444±0.012**	**1.457±0.013**	**1.433±0.012**

* Data are the means over the two growing seasons, 2021 and 2022.
** Plants inoculated with *A. solani*.
*** Before inoculating plants with *A. solani*.
**** Values are the means (mg g^{-1} fresh weight standard deviation) over three replicates from the standard curve of Gallic acid.

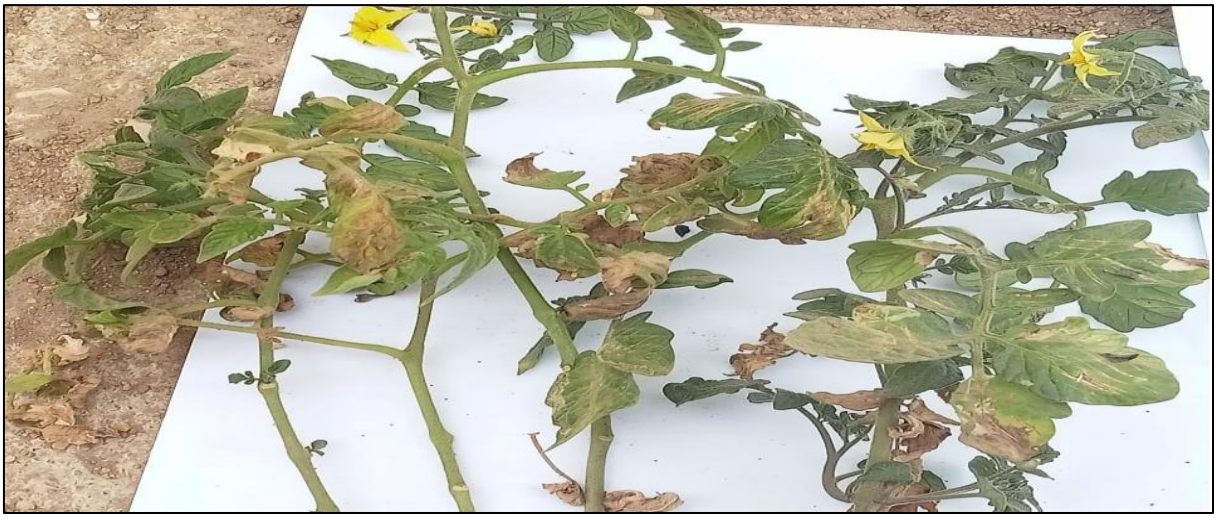

Figure 1a: Symptoms of EB on tomato cv. 844 caused by *A. solani*. Plants infected with EB develop small black or brown spots, usually about 6 to 12 mm in diameter, on older leaves first and stems.

Figure 1b: Symptoms of EB on tomato cv. 844 caused by *A. solani*. Leaf spots are leathery and often have a concentric ring pattern.

DISCUSSION

In the current study, 11 fungal isolates belonging to the genus *Aternaria* were obtained from diseased tomato plants of different cultivars showing EB symptoms collected from different localities in Sohag Governorate. The isolated fungi were identified as *Alternaria alternata* (Fr.) Keissl. (4 isolates) and *Alternaria solani* Sorauer (7 isolates) based on the morphological characteristics of the colony, mycelia, and spores described by Domsch *et al.* (1980). Koch's postulates of the obtained 11 fungal isolates were fulfilled by the pathogenicity tests performed on the tomato cv. 844 under greenhouse conditions in the 2020 growing season. This study demonstrated that all tested isolates of *A. solani* were highly pathogenic to tomato plants, causing typical EB disease symptoms. Isolates No. 11 and 6 caused the highest PDI, followed by isolate No. 7. On the other hand, the tested four isolates of *A. alternata* had weak virulence on tomato plants, causing light blight symptoms with considerable PDI values. The results could be interpreted in light of similar findings reported by Ahmed *et al.* (2009), Kaur *et al.* (2020), Riaz *et al.* (2021), Farooq, Saima *et al.* (2022), Singh and Jangre (2022), and Seemab and Ziau (2023), who found different levels of virulence between the tested fungal isolates of *A. solani,* which may be due to the difference in the genetic structure of each fungal isolate. However, the tomato EB disease was caused by other species of the genus *Alternaria* or other fungal pathogens in different areas worldwide, *A. tomatophila* and *A. grandis* in Brazil (Rodrigues *et al.*, 2010), *A. grandis* in Algeria (Bessadat *et al.*, 2016), *A. phragmospora* in Aswan, Egypt (Abdel-Motaal, Fatma *et al.*, 2020), *A. alternata* in many countries including India and Pakistan (Jewaliya *et al.*, 2021; Riaz *et al.*, 2021) and *Curvularia lunata* as a new causal pathogen of tomato EB disease in Egypt (AbdElfatah, Heba-Alla *et al*, 2021). Biological control offers an environment-friendly alternative to using fungicides for controlling plant diseases. Endophytes refer to a high diversity of microorganisms that inhabit plant host tissues at specific growth stages, and establish mutualism with the host without causing obvious disease symptoms (Petrini, 1991). Many endophytic bacteria and fungi thrive inside plant roots, stems, leaves, or fruits (Aydi Ben Abdallah, Rania *et al*., 2020; Abdelaziz *et al*., 2022; Thulasi Bai *et al*., 2023), and these endophytes colonize plant parts without causing any adverse effects. The use of endophytic microorganisms as a biological control agent has become an attractive, promising, and eco-friendly alternative since this agent could inhibit the vascular progress of the target pathogen, limiting disease incidence and

severity (de Lamo *et al*., 2018; Constantin *et al*. 2019). On many hosts, they act as plant growth-promoting and/or biocontrol agents by direct antagonism through producing secondary metabolites that can serve as antifungal, antibacterial or via the host by triggering induced resistance as well as growth-promoting agents (Constantin *et al*. 2019; Passari *et al*. 2019). In this study, 10 endophytic bacterial and fungal isolates were isolated from the stems and leaves of tomato healthy plants. Based on the Biolog test, the obtained endophytic bacterial isolates were identified as *B. atrophaeus*, *B. subtilis*, *Ps. corrugata*, and *Ps. florsense*. Also, the fungal isolates were identified as *A. nidulans*, *A. terreus*, *P. crustosum*, *T. hamatum*, *T. harzianum*, and *T. koningii* based on the morphological characteristics of the growing colonies, mycelia, and spores described by Domsch *et al.* (1980, 2007). The isolated endophytic microorganisms were then tested for their *in vitro* antagonistic activity against *A. solani* using a dual-culture method. Results showed that all tested isolates endophytic bacterial and fungal isolates varied significantly in their antagonistic activity against *A. solani*. The fungus *T. harzianum* was the most antagonistic microorganism and caused the highest reduction of mycelial radial growth with 7.4 cm and 82.22% of *A. solani*, followed by *B. subtilis* and *T. koningii*. In contrast, *Pe. crustosum* and *Ps. corrugata* caused the lowest reduction of the mycelial radial growth, with (1.1 cm and 12.22%) and (1.2 cm and 13.33%), respectively. These results could be interpreted in light of similar findings reported by Ragab, Asmaa *et al.* (2016), El-Fiki (2017), Ramakrishna *et al.* (2017), Aldiba and Escov (2019), Shoaib, Amna *et al.* (2019), Abdel-Motaal, Fatma *et al.* (2020b), Hatem *et al.* (2022), Abdel-Hamid *et al.* (2023), and Sachdev *et al.* (2023), who tested the same or other bacteria and fungi against the fungus *A. solani in vitro*. The inhibitory effect of these endophytic bacterial and fungal isolates could be attributed to the antibiotics and/or toxic substances secreted, limiting and inhibiting the fungal growth (Winding, 1934; Nadi and Sen, 1953). Under greenhouse and field conditions in the 2021 and 2022 growing seasons, the 4 selected four endophytic bacterial and fungal isolates varied significantly in controlling tomato EB disease, where they decreased the PDI in both growing seasons compared to the control of non-treated plants. *T. harzianum* and *B. subtilis* were the most effective endophytic microorganisms exhibiting the highest reduction in the PDI of tomato EB in 2021 and 2022, followed by *T. koningii*. In contrast, *Ps. florsenses* was the less effective endophytic microorganism and exhibited the lowest reduction in the PDI of EB in 2021 and 2022. These results could also be interpreted in light of similar results reported by Shoaib, Amna *et al.* (2019), Awan, Zoia and Shoaib, Amna (2019), Attia *et al.* (2020), Abdel-Motaal, Fatma *et al.* (2020b), Kulimushi *et al.* (2021), Mohammedi, Aicha *et al.* (2022), Abdel-Hamid *et al.* (2023), and Sun *et al.* (2024), who used the same or other endophytic fungal and bacterial isolates against tomato EB disease. The effective impacts of the four endophytic bacterial and fungal isolates and the disease control could be attributed to their direct strong effect by limiting and inhibiting the pathogens' growth and development in the infected plant tissues (Winding, 1934; Nadi and Sen, 1953) and subsequently suppressing the disease and/or indirect by induction the pathogenesis-related protein with activation the oxidative enzymes such PO, PPO, and catalase as well as increasing the total phenolic contents in plants which play important roles in tomato for inducing disease resistance (Babu, Narendra *et al.*, 2015; El-Fiki, 2017; Awan, Zoia and Shoaib, Amna, 2019; Attia *et al.*, 2020; Abdel-Motaal, Fatma *et al.*, 2020b; Sun *et al.*, 2021; ElSharawy *et al.*, 2023; Rex *et al.*, 2023; Sallam, Nashwa *et al.*, 2023; Li *et al.*, 2024). In the growing seasons of 2021 and 2022 under field conditions, the selected four endophytic bacterial and fungal isolates varied significantly in their affecting plant vegetative growth parameters, where they increased the plant height and shoot fresh and dry weight compared to the control of non-treated plants. *T. harzianum* and *B. subtilis* were the most effective endophytic microorganisms, exhibiting the highest increase in plant height and the shoot fresh and dry weight, followed by *T. koningii* in 2021 and 2022. In contrast, *Ps. florsenses* was the less effective endophytic microorganism and

exhibited the lowest increase in the plant height and shoot fresh and dry weight in 2021 and 2022. These results could also be interpreted in light of similar results reported by Moges *et al.* (2012), Chowdappa *et al.* (2013), Suleiman *et al.* (2017), Manukinda, Adinarayana *et al.* (2018), Awan, Zoia and Shoaib, Amna (2019), Attia *et al.* (2020), Abdel-Motaal, Fatma *et al.* (2020b), and ElSharawy *et al.* (2023), who tested the same or other bacteria and fungi against the fungus *A. solani* under greenhouse and/or field conditions. In this study, the biochemical changes in tomato plants from total protein content, PO and PPO activity, and total phenolic content gradually increased after 2, 4, 8, and 16 days of inoculation with *A. solani* in plants inoculated with the endophytic bacterial and fungal isolates. *T. harzianum* and *B. subtilis* were the most effective endophytic microorganisms, inducing the highest increase in total protein content, PO and PPO activity, and total phenolic content of tomato plants inoculated with *A. solani*, followed by *T. koningii* compared to the control of non-inoculated plants. In contrast, *Ps. florsenses* was the less effective endophytic microorganism and induced the lowest increase in the total protein content, PO and PPO activity, and total phenolic content. These results could also be interpreted in light of similar results reported by Babu, Narendra *et al.* (2015), El-Fiki (2017), Awan, Zoia and Shoaib, Amna (2019), Shoaib, Amna *et al.* (2019), Attia *et al.* (2020), ElSharawy *et al.* (2023), Sallam, Nashwa *et al.* (2023), and Li *et al.* (2024), who used the same or other bacteria and fungi against the fungus *A. solani* and they explained the reason for the effectiveness of these antagonistic endophytes by their ability to stimulate tomato plants to increase them within the tissues. In this regard, numerous endophytic bacteria and fungi against various fungal pathogens have been reported, and they can also increase the activity of defense oxidative enzymes, such as PO and PPO, in the presence of fungal pathogens, these enzymes, such as PO play a vital role in plant defense and resistance. It is well known that the oxidative enzymes PO and PPO play a vital role in the defense mechanism and resistance of the plant towards invading fungal pathogens by oxidation of phenols to toxic quinones, which limit and stop fungal development into plant tissues (Ward, 1986; Quiroga *et al*., 2000; Melo *et al*., 2006; Shimzu *et al*., 2006; Khatun *et al*., 2011). Additionally, the PO enzyme itself was reported to suppress spore germination and mycelial growth of certain fungal pathogens (Joseph *et al*., 1998) and also catalyze the final polymerization step of the lignin synthesis process, which increases the capability of the tissue to lignify, leading to restriction of fungal penetration and infection (Tian *et al*., 2006; Barilli *et al*., 2010).

CONCOLUSSION

The disease largely affects tomato plants' foliage, stems, and fruits, resulting in severe defoliation, decreased yield, and fruit quality. This study aimed to isolate and identify the endophytic microorganisms from tomato plants and evaluate their antagonistic activity against the growth of A. solani in vitro. Also, the efficacy of these endophytic microorganisms in controlling EB disease in the greenhouse and field experiments.

REFERENCES

Abdelaziz, A.M.; Kalaba, M.H.; Hashem, A.H.; Sharaf, M.H.; Attia, M.S. (2022). Biostimulation of tomato growth and biocontrol of *Fusarium* wilt disease using certain endophytic fungi. Botanical Studies 63: 34.

AbdElfatah, Heba-Alla S.; Sallam, Nashwa M.A.; Mohamed, M.S.; Bagy, Hadeel M.M.K. (2021). *Curvularia lunata* as new causal pathogen of tomato early blight disease in Egypt. Mol Biol Rep. 48(3): 3001-3006.

Abdel-Hamid, M.A.M.; Khalil, M.A.; Abbas, M.S.; Abd-El-Moneim, Maisa L.; Soliman, Amira S. (2023). Biological control and molecular differences among some isolates of *Alternaria solani*, the causative agent of early blight in potatoes. Egypt. J. Chem. 66(9): 421-438.

Abdel-Motaal, Fatma; Kamel, N.; El-Zayat, S.; Abou-Ellail, M. (2020a). First report of

Alternaria phragmospora causing early blight disease of *Lycopersicon esculentum* in Egypt. Journal of Biological Studies 3(1): 1-8.

Abdel-Motaal, Fatma; Kamel, Noha; El-Zayt, Soad; Abou-Ellial, M. (2020b). Early blight suppression and plant growth promotion potential of the endophyte *Aspergillus flavus* in tomato plant. Annals of Agricultural Sciences 65: 117-123.

Ahmed, A.A.M.; Atia, M.M.; Abou-Zaid, M.I.; Tohamy, M.R.A. (2009). Pathological studies on tomato early blight. Zagazig Journal of Agricultural Research 33(1): 63-90.

Aldiba, A. and Escov, I. (2019). Biological control of early blight on potato caused by *Alternaria solani* by some bioagents. Advances in Biological Sciences Research 7: 1-5.

Attia, M.S.; El-Sayyad, G.S.; Abd Elkodousd, M.; El-Batal, A.I. (2020). The effective antagonistic potential of plant growth-promoting rhizobacteria against *Alternaria solani*-causing early blight disease in tomato plant. Scientia Horticulturae 266: 109289.

Awan, Zoia A. and Shoaib, Amna. (2019). Combating early blight infection by employing *Bacillus subtilis* in combination with plant fertilizers. Current Plant Biology 20: 100125.

Awan, Zoia A.; Shoaib, Amna; Ali Khan, K. (2018). Variations in total phenolics and antioxidant enzymes cause phenotypic variability and differential resistant response in tomato genotypes against early blight disease. Scientia Horticulturae 239: 216-223.

Aydi-Ben-Abdallah, Rania; Jabnoun-Khiareddine, Hayfa; Daami-Remad, Mejda. (2020). *Fusarium* wilt biocontrol and tomato growth stimulation, using endophytic bacteria naturally associated with *Solanum sodomaeum* and *S. bonariense* plants. Egyptian Journal of Biological Pest Control 30: 113

Babu, rendra A.; Jogaiah, S.; Ito, S.; Nagaraj, A.K.; Tran, L.-S.P. (2015). Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. Plant Science 231: 62-73.

Bais R.K., Ratan, V.; Kumar, S.; Tiwari, A. (2019). Comparative analysis of various strategies for management of early blight of tomato incited by *Alternaria solani* (Ellis and Martin) Jones and Grout. The Pharma Innovation Journal 8: 15-20.

Barilli, E.; Prats, E.; Rubiales, D. (2010). Benzothiadiazole and BABA improve resistance to *Uromyces pisi* (Pers.) Wint. in *Pisum sativum* L. with an enhancement of enzymatic activities and total phenolic content. Eur. J. Plant Pathol. 128: 483-493.

Bessadat, N.; Hamon, B.; Henni, D.E.; Simoneau, P. (2016). First Report of Tomato Early Blight Caused by *Alternaria grandis* in Algeria. Plant Disease 100(2): 533.

Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72: 248-254.

Chaerani, R.; Groenwold, R.; Stam, P.; Voorrips, R.E. (2007). Assessment of early blight (*Alternaria solani*) resistance in tomato using a drop inoculation method, J. Gen. Plant Pathol. 73: 96-103.

Chowdappa, P.; Kumar, S.P. M.; Lakshmi, M.J.; Upreti, K.K. (2013). Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. Biological Control 65: 109-117.

Constantin, M.E.; de Lamo, F.J.; Vlieger, B.V.; Rep, M.; Takken, F.L.W. (2019). Endophyte mediated resistance in tomato to *Fusarium oxysporum* is independent of ET, JA, and SA. Front Plant Sci. 10: 979-992.

de Lamo, F.J.; Constantin, M.E.; Fresno, D.H.; Boeren, S.; Rep, M.; Takken, F.L.W. (2018). Xylem sap proteomics reveals distinct differences between R gene- and endophyte-mediated resistance against *Fusarium* wilt disease in tomato. Front Microbiol. 9: 2977-2989.

Domsch, K.H.; Gams, W.; Anderson, T.H. (2007). Compendium of Soil Fungi. IHW-Verl, Eching, 672 p.

Domsch, K.H.; Gams, W.; Anderson. T.H. (1980). Compendium of soil fungi. Volume 1. Academic Press, London.

Duan, J.-L.; Li, X.-J.; Gao, J.-M.; Wang, D.-S.; Yan, Y.; Xue, Q.-H. (2013). Isolation and identification of endophytic bacteria from root tissues of *Salvia miltiorrhiza* Bge. and determination of their bioactivities. Ann. Microbiol. 63: 1501-1512.

El-Fiki, I.A. (2017). Evaluation of some fungitoxicants for controlling tomato early blight disease. Journal of Phytopathology and Pest Management 4(2): 38-52.

ElSharawy, A.A.; Eid, Azza A.; Ebrahiem, M.Y. (2023). Effectiveness of *Bacillus pseudomycoides* strain for biocontrol of early blight on tomato plants. International Journal of Phytopathology 12(3): 313-326.

FAOSTAT. (2022). Production: Crops and livestock products. In: FAO. Rome. Cited January 2024

Farooq, Saima; Jat, R.R.; Majeed, M.; Nabi, S.U.; Pandit, D.; Khushboo, S.S; Jan, R.; Choudhary, P.; Yadav, M.K. (2022). Morpho-molecular identification of *Alternaria alternata* associated with early blight of tomato (*Solanum lycopersicum* L.). The Pharma Innovation Journal 11(10): 1855-1859.

Gomez, K.A. and Gomez, A.A. (1984). Statistical procedures for agricultural research. 2nd ed. John Willey, New York, 680 pp.

Grigolli, J.F.J.; Kubota, M.M.; Alves, D.P.; Rodrigues, G.B.; Cardoso, C.R.; Henriques da Silva, D.J.; Mizubuti, E.S.G. (2011). Characterization of tomato accessions for resistance to early blight. Crop Breed Appl. Biotech. 11: 174-180.

Hariprasad, P. and Niranjana, S.R. (2009). Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. Plant Soil 316: 13-24.

Hatem, A.; Karpova, N.; Yaderets, V.; Glagoleva, E.; Petrova, K.; Shibaeva, A.; Ovchinnikov, A.; Dzhavakhiya, V. (2022). Inhibition of the growth and development of potato early blight pathogen (*Alternaria solani*) by combining *Penicillium chrysogenum* VKM F-4876D with some strobilurin-, triazole-, and phenylpyrrole-based fungicides. Agriculture 12: 1488.

Hazalin, N.A.; Ramasamy, K.; Lim, S.M.; Wahab, I.A.; Cole, A.L.; Abdul Majeed, A. (2009). Cytotoxic and antibacterial activities of endophytic fungi isolated from plants at the National Park, Pahang, Malaysia. BMC Complementary and Alternative Medicine 9: 46.

Joseph, L.M.; Tan, T.K.; Wong, S.M. (1998). Antifungal effect of hydrogen peroxide and peroxidase of spore germination and mycelial growth of *Pseudocercospora* species. Can. J. Bot. 76: 2119-2124.

Kaur, T.; Yadav, A.N.: Sharma, S.; Singh, N.N. (2020). Diversity of fungal isolates associated with early blight disease of tomato from mid Himalayan region of India, Archives of Phytopathology and Plant Protection 53(13-14): 612-624.

Khatun, S.; Cakilcioglu, U.; Chakrabarti, M.; Ojha, S.; Chatterjee, N.C. (2011). Biochemical defense against die-back disease of a traditional medicinal plant *Mimusops elengi* Linn. Eur. J. Medic. Plant 1: 40-49.

Kofalvi, S. and Nassuth, A. (1995). Influence of wheat streak mosaic virus infection phenylpropanoid metabolism and the accumulation of phenolics and lignin in wheat. Physiol. Mol. Plant Pathol. 47: 365-377.

Kulimushi, S.M.; Muiru, W.M.; Mutitu, E.W. (2021). Potential of *Trichoderma* spp., *Bacillus subtilis* and *Pseudomonas fluorescens* in the management of early light in tomato. Biocontrol Science and Technology 31(9): 912-923.

Li, W.; Sun, L.; Wu, H. *et al.* (2024). *Bacillus velezensis* YXDHD1-7 prevents early blight disease by promoting growth and enhancing defense enzyme activities in tomato plants. Microorganisms 12: 921.

Manukinda, Adinarayana; Anjanappa, Narasegowda, N.C.M.; Devappa, V. (2018). Effect of *Bacillus subtilis* var. *amyloliquefaciens* on growth, yield and control of early blight in tomato (*Solanum lycopersicum* L.). Int. J. Curr. Microbiol. App. Sci. 2018 (Special Issue-7): 656-664.

Maxwell, D.P. and Bateman, D.F. (1967). Changes in the activity of some oxidases in extracts of *Rhizoctonia* infected bean hypocotyls in relation to lesion maturation. Phytopathology 57: 132-136.

Mayee, C.D. and Datar, V.V. (1986). Phytopatholometry Technical Bulletin-1. Marathwad Agric. Univ., Parabhani, India, p. 25.

Melo, G.A.; Shimizu, M.M.; Mazzafera, M. (2006). Polyphenoloxidase activity in coffee leaves and its role in resistance against the coffee leaf miner and coffee leaf rust. Phytochemistry 67: 277-285.

Moges, M.M.; Selvaraj,T.V.; Jebessa, M.T. (2012). Influence of some antagonistic bacteria against early blight (*Alternaria solani* (Ell. & Mart.) Jones & Grout.) of tomato (*Lycopersicon esculentum* Mill.). The African Journal of Plant Science and Biotechnology 6(Special Issue 1): 40-44.

Mohammedi, Aicha; Trachi, S.; Ayad, D.; Messgo-Moumene, S.; Boureghda, H.; Bouznad, Z. (2022). Antagonistic potential of *Trichoderma* spp. evaluated under *in vitro* and *in vivo* conditions against *Alternaria* spp. responsible for early blight of tomato in Algeria. Bulg. J. Agric. Sci. 28(4): 626-635.

Nadi, P. and Sen, G.P. (1953). An antifungal substance from strain of *B. subtilis*. Nature Lond., 172, 4384, p: 871-872. (C.f. Rev. of Appl. Myco. 33: 105).

Passari, A.K.; Upadhyaya, K.; Singh, G.; Abdel Azeem, A.M.; Thankappan, S.; Uthandi, S.; Hashem, A.; Abd Allah, E.F.; Malik, J.A.; Alqarawi, A.S.; Gupta, V.K.; Ranjan, S.; Singh, B.P. (2019). Enhancement of disease resistance, growth potential, and photosynthesis in tomato (*Solanum lycopersicum*) by inoculation with an endophytic actinobacterium, *Streptomyces thermocarboxydus* strain BPSAC147. PLoS One 14: 1-20.

Petrini, O. (1991). Fungal endophytes of tree leaves. Microb. Ecol. Leaves **179**: 197.

Quiroga, M.; Guerrero, C.; Botella, A.; Barcelo, A.; Amaya, I.; Medina, M.I.; Alonso, F.J.; de Forchetti, S.M.; Tigier, T.; Valpuesta, V. (2000). A tomato peroxidase involved in the synthesis of lignin and suberin. Plant Physiol. 122: 1119-1127.

Rabiey, M.; Hailey, E.L.; Roy, R.S.; Grenz, K.; Mahira, A.S.; Al-Zadjali, A.S.M.; Barrett, A.G.; Jackson, W.R. (2019). Endophytes vs tree pathogens and pests: can they be used as biological control agents to improve tree health? Eur. J. Plant Pathol. 155: 711-729.

Ragab, Asmaa M.; El-Habbaa, G.M.; Mohamed, F.G.; El-Fiki, A.I.I.; Abu-Zeid, N.M. (2016). Efficacy of some biotic and abiotic treatments in controlling *Alternaria solani* the causal of tomato early blight disease in vitro and *in vivo*. Annals of Agric. Sci., Moshtohor 54(4): 929-938.

Rahmatzai, N.; Zaitoun, A.A.; Madkour, M.H.; Ahmady, A.; Hazim, Z.; Mousa, M.A.A. (2017). In *vitro* and *in vivo* antifungal activity of botanical oils against *Alternaria solani* causing early blight of tomato. International Journal of Biosciences 10(1): 91-99.

Ramakrishna, A.; Desai, S.; Devi, G.U.; Maheswari, T.U. (2017). Efficacy of different isolates of *Trichoderma* against early blight of tomato. Journal of Pharmacognosy and Phytochemistry 6(5): 1060-1062.

Rex, B.; Gopu, B.; Vinothini, N.; Prabhu, S. (2023). Screening of tomato (*Solanum lycopersicon* L.) genotypes by inducing systemic resistance against early blight disease caused by *Alternaria solani*. Journal of Applied and Natural Science 15(1): 100-106.

Riaz, H.M.; Chohan, S.; Abid, M. (2021). Occurrence of tomato early blight disease and associated *Alternaria* species in Punjab, Pakistan. Journal of Animal & Plant Sciences 31(5): 1352-1365.

Rodrigues, T.T.M.S.; Berbee, M.L.; Simmons, E.G.; Cardoso, C.R.; Reis, A.; Maffia, L.A.; Mizubuti, E.S.G. (2010). First report of *Alternaria tomatophila* and *A. grandis* causing early blight on tomato and potato in Brazil. New Disease Reports 22: 28.

Sachdev, S.; Bauddh, K.; Singh, Rana P. (2023). Prospective of biosurfactant in management of fusarium wilt and early blight of *Lycopersicon esculentum*. Plant Stress 7: 100126. Sallam, Nashwa M.A. (2011). Control of tomato early blight disease by certain aqueous plant

extracts. Plant Pathology Journal 10(4): 187-191.

Sallam, Nashwa M.A., AbdElfatah, H.-A.S.; Khalil Bagy, H.M.M.; Elfarash, A.; Abo-Elyousr, K.A.M.; Sikora, E.J.; Sallam, A. (2023). Exploring the mechanisms of endophytic bacteria for suppressing early blight disease in tomato (*Solanum lycopersicum* L.). Front. Microbiol. 14: 1184343.

Sallam, Nashwa M.A.; AbdElfatah, HA.S.; Dawood, M.F.A. *et al.* (2021). Physiological and histopathological assessments of the susceptibility of different tomato (*Solanum lycopersicum*) cultivars to early blight disease. Eur. J. Plant. Pathol. 160: 541-556.

Seemab, Z. and Ziau, H. (2023). Disease incidence and severity of early blight of tomato caused by *Alternaria solani* in Aligarh District of Uttar Pradesh. Annals of Plant Protection Sciences 31(2): 18-22.

Shimzu, N.; Hosogi, N.; Hyon, G.S.; Jiang, S.; Inoue, K.; Park P. (2006). Reactive oxygen species (ROS) generation and ROS induced lipid peroxidation are associated with plant membrane modifications in host cells in response to AK-toxin from *Alternaria alternata* Japanese pear pathotype. J. Gen. Plant Pathol. 72: 6-15.

Shoaib, Amna; Awan, Zoia A.; Khan, K.A. (2019). Intervention of antagonistic bacteria as a potential inducer of disease resistance in tomato to mitigate early blight. Scientia Horticulturae 252: 20-28.

Suleiman, S.A.; Simon, S.; Babychan, M. (2017). Effect of bioagents and their consortia in the management of early blight disease of potato. International Journal of Agriculture Innovations and Research 5(3): 2319-1473.

Sun, X.; Xinyue, M.A.; Gong, R.; Zhang, D.; Wang, X. (2024). Screening and identification of antagonistic fungi against tomato early blight and research on its pot-plant control effects. Pak. J. Bot. 56(1): 359-365.

Sun, Y.F.; Liu, Z.; Li, H.Y.; Zheng, Z.H.; Ji, C.L.; Guo, Q.; Lai, H.X. (2021). Biocontrol effect and mechanism of *Bacillus laterosporus* Bl13 against early blight disease of tomato. The Journal of Applied Ecology 32(1): 299-308.

Tan, H.L.; Thomas-Ahner, J.M.; Grainger, E.M.; Wan, L.; Francis, D.M.; Schwartz, S.J.; Erdman, J.W.; Clinton, S.K. (2010). Tomato-based food products for prostate cancer prevention: what have we learned, Cancer Metastasis Rev. 29: 553-568.

Thulasi Bai, V.; Seva Nayak, D.; Amrutha, A.V. (2023). Efficacy of endophytic bacteria on plant growth promotion and biocontrol of *Fusarium* wilt in tomato crop. J. Res. Angrau 51(2): 39-51.

Tian, S.; Wan, Y.; Qin, G.; Xu, Y. (2006). Induction of defense responses against *Alternaria* rot by different elicitors in harvested peer fruits. Appl. Microbiol. Biotechnol. 70: 729-734.

Ward, E.W.B. (1986). Biological mechanisms involved in resistance of plants of fungi. In: Baily JA, editor. Biology and molecular biology of plant pathogen interactions. Berlin: Springer-Verlag KG. P.: 107-131.

Winding, R. (1934). Studies on a lethal principle effective in the parasitic action of *Trichoderma lingnorum* on *Rhizoctonia solani* and other fungi. Phytopathology 24: 1153-1179.