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Biological control of *Xanthomonas translucens*, the causal agent of bacterial leaf streak of wheat, by some endophytic fungi

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Abstract

Bacterial leaf streak disease, or black chaff, is one of the most important and damaging diseases of wheat worldwide. The impact of the disease on wheat and cereal production is increasing due to the lack of information and proper management methods to control it. Therefore, to develop an effective approach for managing this disease, the present study assessed the capability of fourteen strains of endophytic fungi to control this disease in laboratory and greenhouse conditions. The laboratory results revealed that volatile compounds produced by endophytic fungi significantly impacted the growth of pathogenic bacteria compared to the control. Furthermore, the greenhouse results indicated that all endophytic fungi significantly reduced the disease severity. A strain of *Pleosporales* sp. showed the highest efficiency in reducing disease severity, with a reduction of 59.03% compared to the infected control. Additionally, *Fusarium moniliforme*, *Trichoderma virens*, *Trichoderma aerugineum*, *Clonostachys rosea*, *Arthrinium arundinis*, and *Epicoccum nigrum* species showed favorable efficiency, reducing disease severity by 50.62%, 49.69%, 48.45%, 47.71%, 41.71%, and 41.67% respectively compared to the infected control. Furthermore, analysis of variance and mean comparison revealed that the effect of endophytic fungi on wheat growth parameters were strain depended. This report is the first to investigate the impact of various endophytic fungi on the control of bacterial leaf streak in wheat. In conclusion, endophytic fungi have great potential to be incorporated into the integrated management of Black Chaff disease and enhance the growth parameters of wheat.

Keywords: Black Chaff, Volatile organic compounds, Disease severity, Biocontrol, Trichoderma, Dark Septate Endophyte

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مهار زیستی *Xanthomonas translucens* عامل بیماری نواری باکتریایی گندم با استفاده از برخی قارچ‌های اندوفیت

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چکیده

مهار زیستی به عنوان یک روش پایدار، دوست‌دار محیط‌زیست و ایمن در سالیان اخیر همواره جهت مدیریت بیماری‌های گیاهی مورد توجه بوده است. بیماری نواری باکتریایی یا سیاه پوشینه از بیماری‌های بذرزاد مهم و خسارت‌زای گندم در سراسر دنیا است که به دلیل فقدان اطلاعات و روش‌های مدیریتی مناسب برای مهار بیماری، تأثیر این بیماری بر تولید گندم و سایر غلات مهم در حال افزایش است. از این رو به منظور دستیابی به راهکاری مناسب جهت مدیریت این بیماری، در پژوهش حاضر توانایی ۱۴ سویه قارچ اندوفیت جهت مهار زیستی این بیماری در آزمایشگاه و گلخانه سنجیده شد. در ازمایشگاه نتایج نشان داد که ترکیبات فرار تولید شده توسط قارچ‌های اندوفیت به صورت معنی‌داری رشد باکتری بیمارگر را نسبت به شاهد کاهش دادند. همچنین نتایج نشان داد که تمامی قارچ‌های اندوفیت در سطح احتمال آماری ۵ درصد به صورت معنی‌داری شدت بیماری را در گلخانه کاهش دادند که از این میان سویه‌ای از *Pleosporales* با کاهش شدت علائم به میزان ۵۹/۰۳ درصد نسبت به شاهد آلوده، بهترین کارائی را داشت. همچنین گونه‌های *Fusarium moniliforme*، *Trichoderma virens*، *Trichoderma aerugineum*، *Clonostachys rosea*، *Arthrinium arundinis* و *Epicoccum nigrum* نیز با عملکرد مطلوبی به ترتیب به میزان ۵۰/۶۲، ۴۹/۶۹، ۴۸/۴۵، ۴۷/۷۱، ۴۶/۷۱ و ۴۱/۶۷ درصد شدت بیماری را نسبت به شاهد آلوده کاهش دادند. علاوه بر این تجزیه واریانس و مقایسه میانگین‌ها، اختلاف معنی‌داری را در اثر برخی سویه‌های قارچی اندوفیت بر صفات رشدی گندم نشان داد. این اولین گزارش در رابطه با اثر سویه‌های مختلف قارچی اندوفیت بر مهار بیماری نواری باکتریایی گندم می‌باشد. درآینده می‌توان با بررسی بیشتر توانایی این سویه‌های قارچی در مهار بیماری و افزایش صفات رشدی گندم به دنبال کاربردی کردن بهترین سویه قارچی اندوفیت در مهار بیماری در مزرعه بود.

کلید واژه: ترکیبات فرار، تریکودرما، سیاه پوشینه، شدت بیماری، قارچ ریسه تیره، مهار زیستی

دبیر تخصصی: دکتر رسول رضائی

Introduction

Strategic crops always play an essential role in the country's food security, economy, and politics. Wheat (*Triticum aestivum* L.) production as a strategic crop has always been the focus of the Ministry of Agriculture, Iran (Tadesse et al., 2016; Javadi et al., 2024). Achieving this goal requires the management of pests and diseases as damaging factors of this valuable product.

Bacterial leaf streak (BLS) or black chaff is one of the most significant and damaging seed-borne diseases of wheat worldwide (Thind, 2019; Sapkota et al., 2020). This disease was first reported in 1936 in the USA and in 1989 in Kerman province, Iran (Bamberg, 1936; Alizadeh & Rahimian, 1989). The symptoms of BLS disease are mainly observed on leaves and spikes. One of the most important symptoms of this disease is narrow, water-soaked streaks on the leaves that later develop into longitudinal yellow to brown streaks with necrotic tissue at the center. Symptoms often develop in the middle of the leaf. Seeds may also be black and shriveled (Duveiller, 1994; Ledman et al., 2020). The disease causes a 40% decrease in wheat yield and a reduced wheat grain quality (Ramakrishnan et al., 2019; Rizvi et al., 2024). The causal agent is *Xanthomonas translucens*, which is gram-negative, rod-shaped (0.5–0.8 × 1.0–2.5 µm), non-sporing, motile by a single polar flagellum, and forms shiny yellow and mucoid colonies on nutrient agar medium.

The pathovars of *X. translucens* were divided into two main groups: Translucens and Graminis (Thind, 2019; Sapkota et al., 2020). The first group, as pathogens of cereals, is divided into four main pathovars, including pv. *cerealis*, pathogenic on wheat, barley, rye, and triticale; pv. *translucens* (= *hordei*) pathogenic on barley; pv. *undulosa* pathogenic on wheat and triticale and pv. *secalis* pathogenic on rye (Vauterin et al., 1995; Duveiller et al., 1997; Sapkota et al., 2020). Nevertheless, in a study using the complete genome sequence and average nucleotide identity (ANI) analysis, all the pathovars of this pathogen were divided into three distinct clades (Goettelmann et al., 2022). The clade Xt-I includes pv. *translucens*, *undulosa*, and *secalis*, and the clade Xt-II includes pv. *cerealis*, and the clade Xt-II includes all pathovars of Graminis (*arrhenatheri*, *graminis*, *phlei*, *phleipratensis*, and *poae*). According to previous studies, pv. *cerealis* can be differentiated genomically from other pathovars in addition to its pathogenicity (Peng et al., 2016; Langlois et al., 2017; Shah et al., 2019). Among the pathovars, pv. *cerealis* is the most prevalent and widespread pathovar in Iran, which damages barley as an important agricultural crop alongside wheat (Alizadeh & Rahimian, 1989).

Biological control as a sustainable, environmentally friendly, and safe method has recently attracted scientists' attention for managing plant diseases (Collinge et al., 2022; Lahlali et al., 2022). Biological agents could inhibit plant pathogens through strategies such as antibiotics, competition, induction of resistance in the host plant, etc. (Legrand et al., 2017; Taheri et al., 2022). In recent years, several studies have been conducted on the effect of rhizobacteria and endophytic bacteria and fungi on the biological control of BLS disease of wheat (Taheri et al., 2022; Afkhamifar, Moslemkhani, Hasanzadeh, & Razmi, 2023; Afkhamifar, Moslemkhani, Hasanzadeh, Razmi, et al., 2023; Niri et al., 2023; Ghasemi et al., 2024; Rizvi et al., 2024).

Endophytes are organisms associated with internal plant tissues or related organs that protect plants against biotic and abiotic stresses (Muhammad et al., 2024). Endophytic fungi protect plants through direct and indirect mechanisms. They have been isolated and identified from all parts of the plant. Endophytic fungi establish a mutualistic relationship with their host plants. These associations promote plant growth, suppressing pathogens and enhancing plant tolerance to abiotic stress (Galindo-Solís & Fernández, 2022; Akram et al., 2023). A group of endophytic fungi known as Dark septate endophytes (DSE) symbiotically exhibit a complex interaction with plant roots, which can be referred to as increasing plant growth and tolerance to biotic and abiotic stresses. Therefore, these fungi have become an attractive option for biological control studies and plant growth promotion in recent years (He et al., 2019; Liu et al., 2022). Well-known endophyte fungi belong to the phyla Ascomycete and Basidiomycete (Wemheuer et al., 2019). Among the most important and common endophytic fungi effective in suppressing plant pathogens and improving plant growth are the genera *Trichoderma*, *Piriformospora*, *Fusarium*, *Alternaria*, *Penicillium*, *Nigrospora*, *Aspergillus*, *Phoma* and *Colletotrichum* (Fontana et al., 2021; Akram et al., 2023; Guzmán-Guzmán et al., 2023; Malarvizhi et al., 2023).

This disease is very important in Kerman, Kermanshah, Lorestan and Hamadan provinces and causes damage every year (Habibian et al., 2021; Hosseini & Marefat, 2021; Alizadeh et al., 2022), but we did not find study on the quantitative and qualitative damage of this disease in Iran. According to the Iranian Research Institute of Plant Protection report, the damage caused by this disease is predicted to be 10%. Most of rust resistance and highly productive cultivars such as Pishgam are susceptible to BLS (Alizadeh et al., 2022). In recent years, due to climate changes, increased rainfall during spring, favorable humidity conditions, and possibly the cultivation of infected seeds, the BLS disease of wheat has spread to different regions of Kermanshah province. Hence, the Department of Plant Protection, Razi University, Kermanshah, first conducted a study by Hosseini and Marafet (2021) to isolate and characterize the disease agent in Kermanshah province. According to the results of that study, the causal agent was identified as *X. translucens* with two pathovars, *undulosa*, and *cerealis* (Hosseini & Marefat, 2021). The present study was conducted to find a suitable approach for managing this disease in the region by performing a biological control with endophytic fungi. It is hoped that the results of this study will help improve our understanding to achieve a suitable management approach for this disease.

Materials and methods

Bacterial pathogen and culture conditions

An isolate of *X. translucens* pv. *cerealis* (Genbank accession number MW193070) was obtained from the Bacterial collection of Department of Plant Protection, Faculty of Agriculture, Razi University, Kermanshah, Iran. The isolate had a regular round, shiny, and transparent yellow colony. It was cultured in NA medium and incubated at 28°C for 3 to 5 days. To preserve it, a suspension of bacteria was prepared in sterile distilled water (SDW) in a 1.5 mL microtube and stored at 4°C (Schaad et al., 2001).

Endophytic Fungi

For biological control of the BLS disease agent, fourteen endophytic fungi belonging to fourteen distinct strains were obtained from the fungal collection of the Plant Protection Department, Faculty of Agriculture, Razi University, Kermanshah, Iran. These isolates have been previously isolated from wheat and barley roots (Shadmani et al., 2018, 2021). Endophytic fungi were cultured in PDA medium and incubated at 25°C for one week. The list of names and information of endophytic fungi used in this study is given in Table 1.

Table 1. List of endophyte fungal strains used in this research

Endophytic fungal Species	Isolate	DSE/non-DSE*	Host	ITS acc. no.	References
Trichoderma virens	RU-TrVi	DSE	Bulk Soil	-	-
Trichoderma harzianum	RU-TrHa	DSE	Bulk Soil	-	-
Trichoderma atroviride	RU-TrAt	DSE	Bulk Soil	-	-
Trichothecium roseum	RU-TrRo	DSE	Tomato	-	-
Trichoderma aerugineum	RU-TrAe	DSE	Bulk Soil	-	-
Arthrinium arundinis	RU-ArAr	non-DSE	Wheat	-	-
Fusarium tricinctum	RU-FuTr	non-DSE	Barley	KX343029	(Shadmani et al., 2021)
Fusarium moniliforme	RU-FuMo	non-DSE	Barley	KX343028	(Shadmani et al., 2021)
Alternaria sp.	TBR8	DSE	Barley	KX061185	(Shadmani et al., 2021)
Alternaria sp.	tw6-1	DSE	Wheat	KX061188	(Shadmani et al., 2021)
Microdochium bolleyi	RU-MiBo	DSE	Barley	KX343031	(Shadmani et al., 2018)
Epicoccum nigrum	RU-EpNi	non-DSE	Barley	-	(Shadmani et al., 2021)
Clonostachys rosea	RU-ClRo	non-DSE	Barley	-	(Shadmani et al., 2021)
Pleosporales sp. Tw24	RU-Pleosp	DSE	Wheat	KX061191	(Shadmani et al., 2021)

***** Dark septate endophyte

In Vitro Biocontrol assay

Two-partite I-plates were used to investigate the effect of volatile organic compounds produced by endophytic fungi on inhibiting the growth of pathogenic bacteria. PDA medium was poured into one part of the I-plate in sterile conditions, and endophytic fungi were cultured and incubated at 25°C. On the other part, 500 µL of the bacterium pathogen was cultured in 10 mL of Nutrient Broth (NB) and incubated in a shaker incubator at 150 rpm at 28°C for 72 hours. To prevent fungi cross-contamination, NB medium was treated with the fungicide chlorothalonil (1 mg 10 mL^{-1}). The lids of the Petri dishes were sealed with parafilm to prevent the release of volatile compounds. SDW was used instead of endophytic fungi in the control treatments. After 24 hours, using a spectrophotometer, the optical density (OD) of the bacterial suspension was measured at a wavelength of 600 nm and compared with the control treatment (Sharifi & Ryu, 2016). The inhibition rate of pathogenic bacteria growth was calculated using formula 1. In this formula, GIR (%) represents the percentage of inhibition of the growth of pathogenic bacteria, OD control refers to the concentration of growth of pathogenic bacteria in the control treatment, while OD treatment indicates the concentration of growth of pathogenic bacteria in treatments with endophyte fungi (Huang et al., 2017).

$$\text{Growth Inhibition Rate } (\%) = \frac{\text{OD control - OD treatment}}{\text{OD control}} \times 100 \qquad (1)$$

Greenhouse experiments

Bacterial pathogen and endophytic fungi preparation

A healthy wheat seed substrate was used to prepare endophytic fungi inoculum. The wheat seeds were washed, and 200 g of them were transferred into a 500 mL Erlenmeyer flask and autoclaved three times at 24-hour intervals. Then, some pieces (5-mm disc) of fresh culture of endophytic fungi were added to each Erlenmeyer flask and incubated at 25°C for one month until the fungus completely colonized the seeds.

To prepare the bacterial pathogen inoculum, it was streaked on a NA medium and incubated at 28°C for 48 hours. The bacterial inoculum was prepared by suspending bacterial cells in sterile normal saline (8.5 g L^{-1} of NaCl salt). The concentration of the resulting suspension was measured using a spectrophotometer at a wavelength of 600 nm, which had a concentration equal to 10^8 cells mL^{-1} (OD_{600}=0.1).

In planta biocontrol assay

A greenhouse test was conducted to investigate the effect of endophytic fungi on the suppression of disease severity and promotion of the growth parameters of wheat in the greenhouse condition in a completely randomized design with 16 treatments (14 endophytic fungi, one healthy control, and one infected control) in three replicates (Table 2). The plastic pots (1.5 L volume) were filled with a sterilized mixture of soil and perlite in a ratio of 2:1. Wheat seeds, Pishgam cultivar, were obtained from The Agricultural and Natural Resources Research Center, Kermanshah, Iran. The seeds were surface-disinfected by immersing them in 70% ethanol and then 0.5% sodium hypochlorite (1 min for each) at room temperature and thoroughly washed with sterile distilled water five times. Then, the sterile seeds were planted in pots. At planting time, 20 grams of colonized seeds with endophytic fungi were mixed with the soil in each pot. After the wheat reached the stage of 3 to 5 leaves, the pathogenic bacterium suspension prepared with a concentration of 10^8 cells mL^{-1} was sprayed on the plant leaves using a spray. The wheat plants were covered with transparent plastic bags for 24 hours to maintain their humidity. The healthy control treatment was inoculated using SDW. The treatments were kept in a greenhouse with a temperature of 28°C and were irrigated daily using water with 100 ppm of a complete fertilizer (NPK+TE, 18-18-18).

Measurement of Disease Severity and Wheat Growth Parameters

After 15 days of pathogen inoculation and the appearance of disease symptoms in the treatments, the disease severity was recorded using the standard scale of BLS disease index shown in Figure 1 (Duveiller, 1994; Duveiller et al., 1997). Formula 2 was used to calculate the percentage of disease severity (DS), and Formula 3 was used to calculate the reduction of disease severity compared to the infected control (Ji et al., 2008; Niri et al., 2023). In this formula, based on symptoms assessment under greenhouse, the disease index was considered from 1 to 7 (Figure 1), where scale 1 was equivalent to leaves with 1% severity of symptoms and scale 7 was considered to be equivalent to 100% severity of symptoms in leaves. After evaluating the disease severity index, the plants were slowly removed from the pots. After washing the roots, various growth parameters were measured, including root volume, root wet and dry weight, and shoot wet and dry weight. The resulting data were recorded and subjected to statistical analysis.

$$\text{Disease Severity (DS) (\%)} = \frac{\sum_{i=1}^{n} (X \times K)}{N \times 7} \times 100 \quad (2)$$

In this formula, X is the total number of graded leaves, K is each leaf's disease severity grade (1 to 7), and N is the total number of graded leaves.

$$\text{Reduction of Disease Severity (\%)} = \frac{\text{DS control - DS treatment}}{\text{DS control}} \times 100 \quad (3)$$

Statistical analysis

All the tests were performed in a completely random design, and three repetitions were considered for each treatment. Analysis of variance (ANOVA) procedure in SAS (version 9.3) was used to analyze the data obtained from laboratory and greenhouse tests (Moodie & Johnson, 2022). Mean comparison was performed using Duncan’s Multiple Range Test. GraphPad Prism 8 software was used to draw the graphs.

Results

In vitro antibacterial activities of endophytic fungi on *X. translucens* pv. *cerealis*

13 out of 14 endophytic fungi significantly reduced the growth of pathogenic bacteria compared to the control treatments at the

probability level of 5% (Figure 2). The volatile compounds from *F. tricinctum* had the most significant impact on pathogen growth, resulting in a 50.18% inhibition rate. Among the endophytic fungi, the lowest effect on pathogen growth inhibition was recorded in *A. arundinis* species. No statistically significant difference existed between this species and the control treatments (Figure 2).

Table 2. List of treatments in greenhouse experiments.

Treatments	Code
Infected Control (with pathogen)	**Infected**
Non-Infected Control (without pathogen and endophytic fungi)	**Healthy**
Trichoderma virens + *X. t* pv. *cerealis*	**TrVi**
Trichoderma harzianum + *X. t* pv. *cerealis*	**TrHa**
Trichoderma atroviride + *X. t* pv. *cerealis*	**TrAt**
Trichoderma aerugineum + *X. t* pv. *cerealis*	**TrAe**
Trichothecium roseum + *X. t* pv. *cerealis*	**TrRo**
Fusarium tricinctum + *X. t* pv. *cerealis*	**FuTr**
Fusarium moniliforme + *X. t* pv. *cerealis*	**FuMo**
Alternaria sp. TBR8 + *X. t* pv. *cerealis*	**AltTBR8**
Alternaria sp. tw6-1 + *X. t* pv. *cerealis*	**Altw6**
Microdochium bolleyi + *X. t* pv. *cerealis*	**MiBo**
Epicoccum nigrum + *X. t* pv. *cerealis*	**EpNi**
Arthrinium arundinis + *X. t* pv. *cerealis*	**ArAr**
Clonostachys rosea + *X. t* pv. *cerealis*	**ClRo**
Pleosporales sp. tw24 + *X. t* pv. *cerealis*	**Pleosp**

Figure 1. The standard disease assessment key shows percentages of leaf surface covered by bacterial leaf streak in bread wheat (Duveiller, 1994).

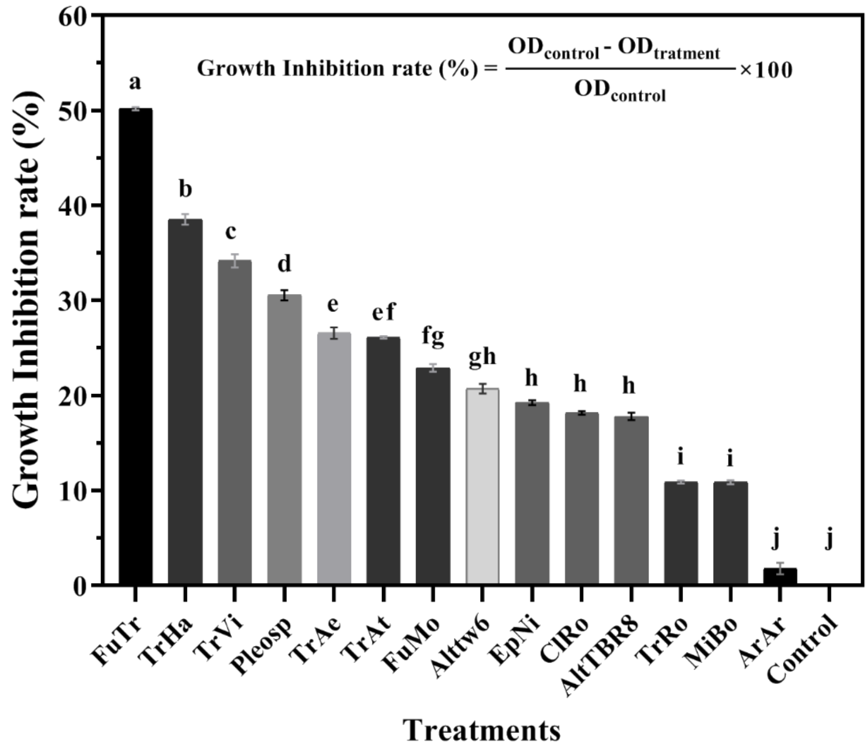

Figure 2. The effect of volatile compounds of endophytic fungi on the population growth inhibition of *Xanthomonas translucens* pv. *cerealis* in NB medium *in vitro*. The means were compared at the level of 5% probability using Duncan's multiple range test. Difference between means with common letters are not statistically significant.

Effects of endophytic fungi on disease severity in greenhouse

The role of endophytic fungi in reducing disease severity was also determined in this study. When *X. translucens* pv. *cerealis* was co-inoculated with each endophytic fungus, the lengths and the number of lesions were significantly shorter compared to leaves that were inoculated solely with this phytopathogen ($P \leq 0.05$). The healthy control treatment, inoculated with SDW, showed no symptoms. The results of investigating the effect of endophytic fungi on controlling the disease and reducing the severity of symptoms of BLS disease of wheat are shown in Figure 3 and Table 3. All of the endophytic fungi used in this study significantly reduced disease severity compared to the infected control at the 5% probability level. Among the endophytic fungi, *Pleosporales* sp. has been found to provide the highest level of disease suppression. It was found that the severity of the symptoms decreased by 59.03% compared to the infected control (Table 3). Moreover, *F. moniliforme*, *T. virens*, *T. aerugineum*, *C. rosea*, *A. arundinis*, and *E. nigrum* reduced disease severity by 50.62, 49.69, 48.45, 47.71, 46.71, and 41.67% respectively compared to the infected control (Table 3). The lowest disease suppression was related to *T. roseum*, which decreased the disease index by 22.12% compared to the infected control. Figure 4 clearly displays the varying percentages of disease severity symptoms observed in the sample of wheat leaves.

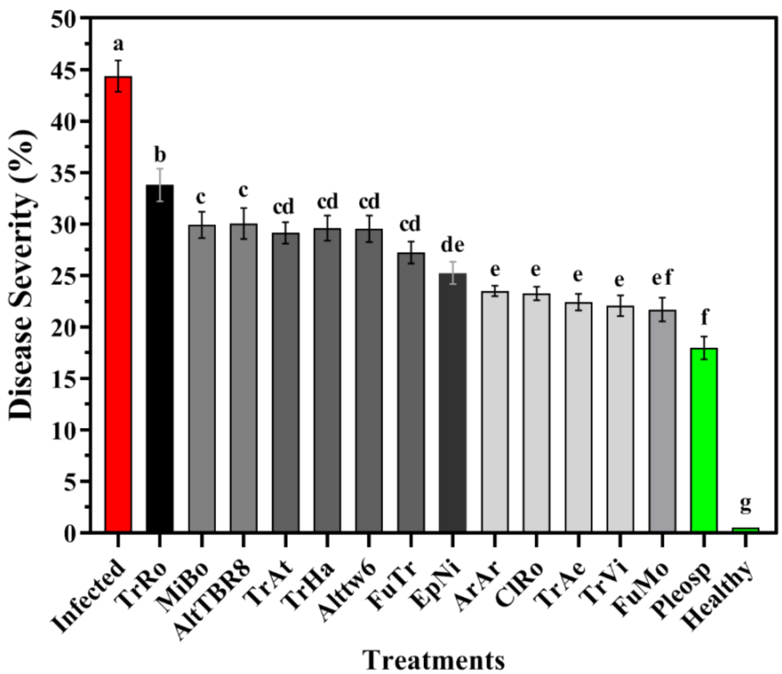

Figure 3. The effect of endophytic fungi on the bacterial leaf streak disease severity caused by *Xanthomonas translucens* pv. *cerealis* in the greenhouse. The means were compared at the level of 5% probability using Duncan's multiple range test. Difference between means with common letters are not statistically significant.

Effect of treatments on wheat growth parameters

In addition to reducing the BLS disease severity, the endophytic fungi used in this study significantly improved wheat growth parameters compared to the infected control (Figures 5 and 6). In examining the wet and dry weight of shoot, 12 out of 14 endophytic fungi showed a significant difference compared to the infected control. However, the two species, *T. virens*, and *T. aerugineum* did not have a significant difference compared to the infected control (Figure 6 - A and B). Two species, *E. nigrum* and *A. arundinis*, showed a significant difference in root wet weight parameter compared to the healthy and infected control (Figure 6-C). In root volume parameter, endophytic fungi *E. nigrum*, *A. arundinis*, and *F. tricinctum* significantly increased the root volume compared to the infected and healthy control (Figure 6-E). In Figure 5, examples of pots with different sizes of growth parameters are displayed. This image shows the difference in the size and volume of the shoot and root in the sample of various treatments (Figure 5).

Correlation among traits evaluated in laboratory and greenhouse

All the evaluated plant growth parameters had a high correlation with each other (Table 4). Among these traits, shoot wet and dry weight had a very significant correlation (Pearson correlation coefficient 0.956 and probability of significant difference 0.001). The correlation between root wet weight and root volume was very high, but these two traits had a lower but significant correlation with root dry weight (Bashan et al., 2017). None of the growth traits of wheat significantly correlated with disease control under greenhouse conditions. This indicates that endophytic fungi use different mechanisms to increase plant growth and suppress the pathogen. However, the data collection was done in the early growth stages of wheat and before the appearance of spikes, so the disease symptoms did not have much effect on the growth parameters of wheat. Interestingly, the growth inhibition of the pathogen in laboratory conditions did not significantly correlate with the suppression of the pathogen in greenhouse conditions.

Table 3. The effect of endophytic fungi on reducing the severity of bacterial leaf streak of wheat caused by *Xanthomonas translucens* pv. *cerealis* compared to the infected control

Treatments	Reduction of disease severity compared with control (%)
Infected Control	-
Trichoderma virens + *X. t* pv. *cerealis*	49.69e
Trichoderma harzianum + *X. t* pv. *cerealis*	34.79cd
Trichoderma atroviride + *X. t* pv. *cerealis*	33.35cd
Trichoderma aerugineum + *X. t* pv. *cerealis*	48.45e
Trichothecium roseum + *X. t* pv. *cerealis*	22.12b
Fusarium tricinctum + *X. t* pv. *cerealis*	37.25cd
Fusarium moniliforme + *X. t* pv. *cerealis*	50.62ef
Alternaria sp. TBR8 + *X. t* pv. *cerealis*	31.66c
Alternaria sp. tw6-1 + *X. t* pv. *cerealis*	35.20cd
Microdochium bolleyi + *X. t* pv. *cerealis*	31.47c
Epicoccum nigrum + *X. t* pv. *cerealis*	41.67de
Arthrinium arundinis + *X. t* pv. *cerealis*	46.71e
Clonostachys rosea + *X. t* pv. *cerealis*	47.71e
Pleosporales sp. tw24 + *X. t* pv. *cerealis*	59.03f

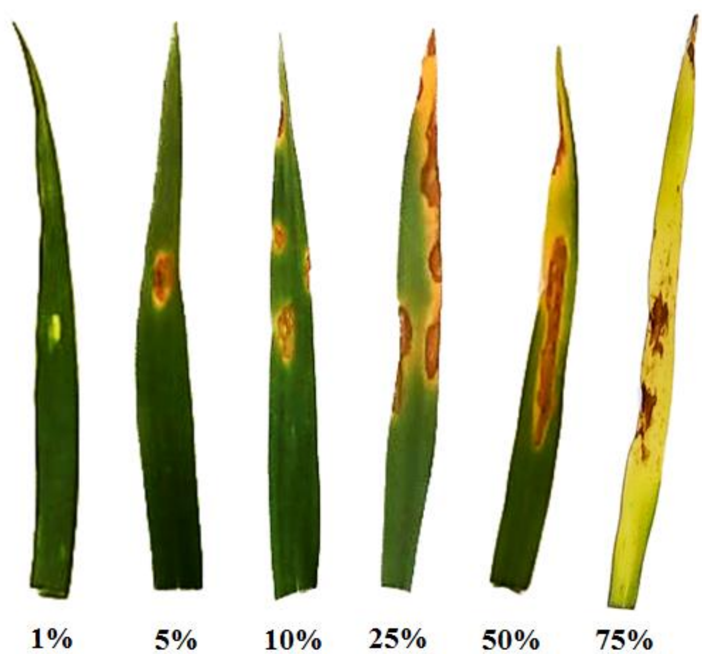

Figure 4. Different symptoms of bacterial leaf streak disease of wheat under greenhouse conditions in the current study.

Figure 5. Shoots (A) and roots (B) in the samples of the treatments applied in the greenhouse. In the current illustration, it is apparent that there is a difference in the size and volume of the shoot and root between healthy and infected treatments.

Discussion

In recent years, the increased spread and damage of BLS disease in wheat can be attributed to favorable environmental conditions, such as high humidity and warm weather, and the planting of infected seeds. One major challenge in controlling this disease is the susceptibility of high-yielding and rust-resistant cultivars to this pathogen. Therefore, in this research, the biological control of BLS disease of wheat was investigated using the ability of some endophytic fungi. The results showed that several species of endophytic fungi used in this research were able to significantly reduce the severity of the disease. The use of endophytic fungi and bacteria for biological control has emerged as a new and fascinating technique in recent years (Fontana et al., 2021; Collinge et al., 2022; Guzmán-Guzmán et al., 2023; Muhammad et al., 2024). The present study is the first to investigate the effect of endophytic fungi, including *Trichoderma*, *Fusarium*, *Alternaria*, *Clonostachys*, *Microdochium*, *Epicoccum*, *Trichothecium*, and *Arthrinium*, on controlling BLS disease of wheat.

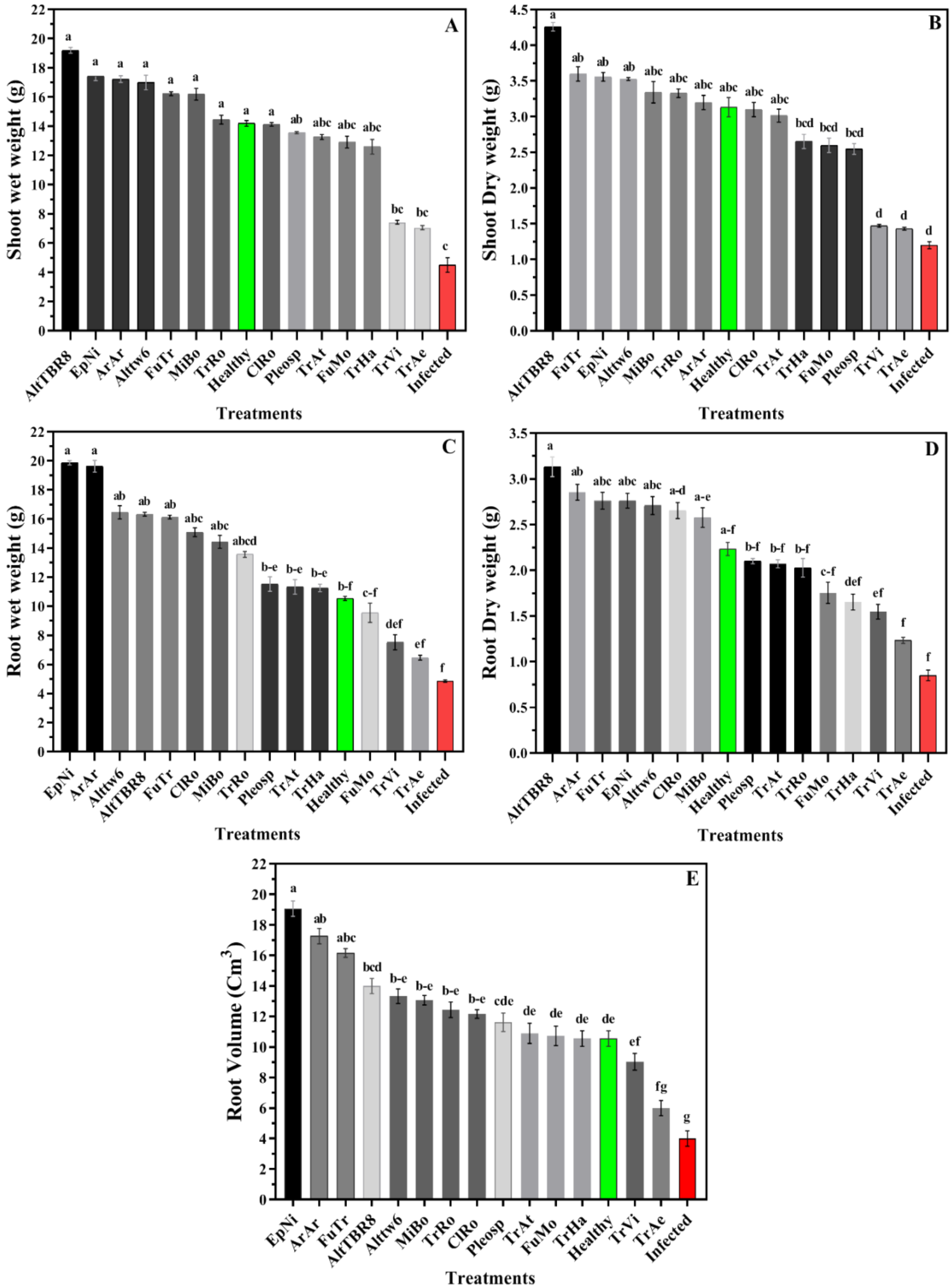

Figure 6. The effect of endophytic fungi on the growth parameters of wheat in the presence of *Xanthomonas translucens* pv. *cerealis* in the greenhouse. A) Shoot wet weight, B) Shoot dry weight, C) Root wet weight, D) Root dry weight, E) Root volume. The means were compared at the level of 5% probability using Duncan's multiple range test. Difference between means with common letters are not statistically significant.

Table 4. Correlation between growth parameters, disease severity index, and inhibition of bacteria pathogen population in laboratory conditions

	SWW	RWW	SDW	RDW	RV	Disease
SWW	1					
RWW	0.761**	1				
SDW	0.956**	0.721**	1			
RDW	0.693**	0.563**	0.694**	1		
RV	0.689**	0.960**	0.648**	0.488**	1	
Disease	-0.093ns	0.092ns	0.013ns	-0.086ns	0.130ns	1
In vitro	0.305*	0.332*	0.237ns	0.293ns	0.121ns	

ns, *and **; indicates no significant correlation, correlation at the probability level of 5% and 1%, respectively.

In 2023, the effect of an endophytic fungus, *Piriformospora indica*, on controlling BLS disease was investigated for the first time (Niri et al., 2023). However, most initial studies focused on controlling the disease using epiphyte antagonistic bacteria (Stromberg et al., 2000). In a study conducted by Niri et al. in 2023, the endophytic fungus *P. indica* reduced the severity of BLS disease by 62% compared to the infected control. In the current study, the endophytic fungi *Pleosporales* sp., *F. moniliforme*, *T. virens*, *T. aerugineum*, *C. rosea*, *A. arundinis*, and *E. nigrum* have reduced the severity of the disease by 59.03, 50.62, 49.69, 48.45, 47.71, 46.71 and 41.67% compared to the infected control, respectively (Table 3). In other studies that evaluated the effect of some bacterial species on controlling BLS disease, *Paenibacillus polymxa* species reduced the severity of the disease by 68% and *Stenotrophomonas maltophilia* by 59% compared to the infected control (Taheri et al., 2022; Ghasemi et al., 2024). In the treatments with endophytic fungi, necrotic and water-soaked lesions were much smaller than the infected control. By reducing the necrotic lesions on the leaf surface, which are the main symptoms of the disease, photosynthetic levels will increase compared to the diseased leaf. This will result in an increase in both the quantity and quality of the wheat. Previous studies have revealed that biological control agents cannot prevent 100% of disease severity and can only reduce it to varying degrees. Therefore, since this disease primarily damages leaf tissue, reducing necrotic tissue can minimize the overall damage caused by the disease.

Among fungal biological control agents, the genus *Trichoderma* has been the most studied and exploited in recent years (Sharma et al., 2023). Due to its ability to produce various enzymes, antibiotics, induce plant resistance, and inhibit various pathogens, this fungus has become the most researched biopesticide and biofertilizer (Waghunde et al., 2016; Sharma et al., 2023). This study evaluated the effects of several species of *Trichoderma* on the control of BLS disease. *T. virens*, *T. aerugineum*, *T. harizanum*, and *T. atroviride* reduced the severity of the disease by 49.69, 48.45, 34.79 and 33.35% compared to the infected control. In a study, *T. atroviride*, by producing secondary metabolites showed high efficiency in inhibiting the bacterial pathogen *Xanthomonas campestris* pv. *campestris* (Papaianni et al., 2020). Additionally, in another report, the bioactive compounds produced by *T. harizanum* species could inhibit the virulence factors of bacterial pathogens, such as secretion systems and quorum sensing. They showed high efficiency in inhibiting bacterial pathogens *Xanthomonas campestris*, *Clavibacter michiganensis*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Anwar & Iqbal, 2017).

Dark septate endophytes (DSE), which are a diverse group of Ascomycetes fungi from the roots of hundreds of plant families, help plants with mechanisms such as increasing the content of chlorophyll, the rate of photosynthesis and the production of plant hormones, and inducing the defense mechanisms (Malicka et al., 2022). In the present study, in addition to *Trichoderma*, some strains from two different genera of dark septate fungi, including *Alternaria* sp. and *Pleosporales* sp., were able to significantly reduce the severity of the disease compared to the control. Furthermore, *M. bolleyi* species, as one of the most well-known dark septate fungus, was able to reduce the severity of the disease by 31.47% compared to the infected control. In this study, in addition to inhibiting the disease severity, these dark septate endophytes increased the growth parameters of the wheat compared to the infected control. In past studies, *M. bolleyi*, a successful endophytic fungus in the roots of various plants, such as wheat and barley, has demonstrated the ability to inhibit plant pathogens (Comby et al., 2017; Shadmani et al., 2018).

In the present study, three species of endophytic fungi, *C. rosea*, *A. arundinis,* and *E. nigrum,* reduced the disease severity compared to the infected control by 47.74, 46.71 and 41.67%, respectively. They also significantly increased root wet weight and volume compared to infected and healthy controls. Plant growth promotion is considered one of the main mechanisms of endophytic fungi to control plant diseases. Several studies have shown that bacterial and fungal endophytes enhance host plant growth by biosynthesis of plant hormones, nitrogen fixation, phosphate solubilization, and production of siderophores (Kandel et al., 2017; Mehta et al., 2019; Carrie et al., 2023). In past studies, *C. rosea* has inhibited the growth of pathogens by producing secondary metabolites and affecting plant growth (Fatema et al., 2018; Sun et al., 2020; Zhai et al., 2016). Past reports have also shown that *Epicoccum* species, as a saprophytic and endophytic fungus, exploit various metabolites to inhibit plant pathogens and increased the growth parameters of various plants (Taguiam et al., 2021; Li et al., 2022).

In this study, *F. moniliforme* and *F. tricinctum* species reduced the disease severity compared to the control by 50.62% and 37.25%, respectively. Moreover, *F. tricinctum* not only significantly increased the growth parameters of wheat compared to the infected control, but it also increased the root volume significantly compared to the healthy control. *Fusarium* species, in addition to being present in various studies as a prevalent endophytic fungus that improves the growth of plants, they can inhibit various plant pathogens by producing antimicrobial metabolites and volatile compounds (Zhang et al., 2014; Carrie et al., 2023).

Volatile organic compounds (VOCs) are compounds with low molecular weight that are the result of secondary metabolism of living organisms such as bacteria and fungi. As a chemical language, they play important roles in the interactions between plants and microbes (Yuan et al., 2017; Sharifi & Ryu, 2018a). Induction of systemic resistance, inhibition of fungal and bacterial pathogens, plant growth promotion, and increasing the tolerance of plants against biological stresses are among the beneficial roles of volatile compounds produced by microorganisms (Veselova et al., 2019; Raza & Shen, 2020; Sharifi et al., 2021). In this study, the possible effect of volatile compounds produced by endophytic fungi on the growth of bacterial pathogens was investigated in laboratory conditions using a two-partite I-plate. As can be seen from the results, 13 of the 14 endophytic fungi significantly reduced pathogen growth in the NB medium compared to the control. Due to this, the production and release of volatile compounds by endophytic fungi can be one of their mechanisms to inhibit pathogen growth and further control the severity of the disease.

Examining the correlation between disease severity in the greenhouse and inhibition of bacterial population growth in the laboratory showed no significant correlation between these two factors (Table 4). Biological control agents use several mechanisms to inhibit the pathogen in the plant, which either cannot be tested in the laboratory, like the induction of resistance, or the activation of those mechanisms depends on sensing a specific environmental signal received from the host or the pathogen (Sharifi & Ryu, 2018b). Considering that the strains used in this research are endophytes and colonizers of wheat roots, there is no direct contact between the pathogen and the endophyte fungus in plant conditions, and indirect methods play a more important role in controlling the disease. Therefore, screening in the laboratory does not have much correlation with the efficiency of the biological control agent in nature, and sometimes, it causes the loss of efficient isolates. In addition, there was no significant correlation between the effectiveness of the isolates used in this research in controlling the disease and increasing growth parameters (Table 4). The two species *Trichoderma virens* and *Trichoderma aerugineum* caused a reduction of 49.69 and 45.48 disease severity, but in most of the growth parameters, they did not differ significantly from the infected control. In contrast, the isolates of *Alternaria* sp. TBR8 and *Epicoccum nigrum* were effective isolates in improving plant growth parameters but were moderate to weak in disease suppression.

Climatic changes, particularly rainfall during the warm months of May and June, have caused the spread of BLS or black chaff disease in cereals. The time of the outbreak of this disease is such that it is not possible to enter the field with spraying machines, and existing copper bactericides do not have an acceptable effect in controlling the disease. However, our results showed that biological control agents, especially endophytic fungi, can be a promising approach to managing the disease. The best strain of current research was able to reduce approximately 60% of the severity of the disease. Although there were other strains with lower biocontrol abilities, they could increase the growth parameters effectively. The variety of mechanisms and the ability of isolates to control disease and plant growth have made researchers interested in the combined use of these strains under the title of microbial consortia. If their compatibility with each other is proven, the consortia of strains will guarantee the possibility of their use in different conditions and ensure their efficiency (Hussein et al., 2018; Hozhabri et al., 2023).

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Conflict of interests

The authors declare that they have no conflict of interests.

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