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RESEARCH

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Promising biological agents represented in *Bacillus velezensis* 33RB and *Aspergillus niger* 46SF endophytic isolates for controlling *Populus tomentosa* wilt and anthracnose diseases

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Abstract

Background: Poplar fungal infections are difficult to control and result in severe economic loss. As a viable alternative to chemical pesticides, biocontrol is an effective safe method for disease control.

Results: Inhibitory activity of *Bacillus velezensis* 33RB and *Aspergillus niger* 46SF was evaluated against numerous phytopathogens. The bacterial strain displayed the highest inhibitory activity toward *Colletotrichum gloeosporioides* BJ02 and *Fusarium oxysporum* 20RF (61.2 and 49.4%, respectively). Also, the maximum inhibitory activity of *A. niger* 46SF was exhibited (75.51 and 70.83%) against *C. gloeosporioides* BJ02 and *F. oxysporum* 20RF, respectively. The minimum volume (6.25 ml) of sterilized cultural filtrate of bacterial and fungal strains significantly inhibited the growth of *C. gloeosporioides* BJ02 by 73.3 and 83.3%, respectively, and *F. oxysporum* 20RF reached 40.4 and 78.8%, respectively. *B. velezensis* 33RB and *A. niger* 46SF displayed the highest inhibition toward *C. gloeosporioides* BJ02 and *F. oxysporum* 20RF at neutral pH and pH 5, respectively. Moreover, the highest inhibitory activity of *B. velezensis* 33RB and *A. niger* 46SF was achieved at 37 °C and 28 °C, respectively. Pathogenicity tests on sterilized detached leaves indicated that these isolates could potentially affect anthracnose and fusarium wilt diseases. Several secondary bioactive metabolites that assured the biocontrol efficacy of tested microbes were detected by liquid chromatography–mass spectrometry (LC–MS). The most detectable compounds included organic acids such as fumaric, DL-malic, citric, isobutyric, and glutamic acids. Also, numerous fatty acids such as lauric, linoleic, oleic, stearic, and myristic acids with diverse biological functions, including antimicrobial properties, were determined.

Conclusions: *Bacillus velezensis* 33RB and *A. niger* 46SF were potential alternatives to chemical pesticides as biological control agents for the phytopathogens *C. gloeosporioides* BJ02 and *F. oxysporum* with environmentally friendly and sustainable properties.

Keywords: *Populus tomentosa*, Biological control, Phytopathogenic fungi, LC–MS/MS analysis

Background

The fast-growing Chinese white poplar trees (*Populus tomentosa* Carr.) offer significant ecological and economic benefits and high-quality wood for bioenergy production (Du et al. 2012). These trees are affected by severe diseases. Poplar anthracnose is caused by the fungus, *Colletotrichum gloeosporioides*, which infects the leaves and frequently causes premature defoliation

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(Huang et al. 2020). *Fusarium* spp. have also been linked to reddish-purple staining, necrosis, swelling, and the development of distinct cankers in poplar (Kwaśna et al. 2017). Chemical agents are used to treat these diseases; however, they pollute the ground and soil water reservoirs and cause an undesirable accumulation of chemical residues in the food chain (Barák 2017). Studies have focused on circumventing these consequences by reducing or replacing the use of chemical pesticides.

For these issues, beneficial microbes have been increasingly used as biological control agents. Endophytic bacteria can substitute chemical fertilizers and pesticides and increase plant output and resistance to biotic and abiotic stress (Shah et al. 2021). Their advantages include their ability to colonize internal plant tissues (Fadiji and Babalola 2020), control plant disease (Zhang et al. 2015), enhance crop growth (Faria et al. 2013) and resistance (Pavlo et al. 2011), and promote organic pollutant degradation (Zhang et al. 2014).

Antagonistic strains can control phytopathogenic fungi via mycoparasitism, competition, generation of antibiotics, and stimulation of defense responses (Jinal and Amaresan 2020). Endophytes produce many bioactive metabolites such as alkaloids, phenolic acids, steroids, quinones, saponins, terpenoids, and tannins, making them promising anticancer, antituberculosis, antimalarial, antiviral, anti-inflammatory, and antidiabetic agents. These bioactive substances also make the host plants resistant to biotic and abiotic stress (Fadiji and Babalola 2020).

Pageni et al. (2014) indicated that biocontrol bacterial strains such as *Bacillus*, *Aureobacterium*, *Paenibacillus*, *Pseudomonas*, *Phyllobacterium,* and *Burkholderia* produce various metabolic compounds such as lytic enzymes and antibiotics, which inhibit the growth of fungal phytopathogens, including *Fusarium oxysporum, Sclerotium rolfsii, Rhizoctonia solani,* and *Verticillium dahliae. Bacillus* species were frequently instead of chemical pesticides (Kamali et al. 2022). Additionally, fungal endophytes can protect the host plant from disease by reducing the harmful effect of phytopathogens (Ganley et al. 2008). It was reported that *Aspergillus* species can synthesize volatile and/or nonvolatile metabolites against numerous pathogenic fungi, including *Pythium* spp, *Sclerotinia sclerotiorum,* and *F. oxysporum* (Bhattacharyya and Jha 2012).

Recently developed techniques facilitated the investigation of plant growth-promoting rhizobacteria (PGPR) and endophytes for biocontrol applications (Sellami et al. 2021). The genomes of PGPR or endophytes were thoroughly studied using metagenomics, while their secretomes were extensively analyzed using LC–MS technology. These studies clarified the role of PGPR and endophytes as biocontrol agents against plant infections (Bailly and Weisskopf 2017).

The present study aimed to delineate the biocontrol activities of the endophytes against various plant pathogens, including *F. oxysporum*20FR and *C. gloeosporioides* BJ02, and identify several secondary antifungal metabolites using LC–MS spectroscopy.

Methods

An overview of the work done in this study is depicted in Fig. 1.

Endophytic microbes

Five bacterial strains, namely *Enterobacter tabaci* 24RB (MN540932.1), *Bacillus velezensis* 33RB (MN559965.1), *Bacillus megaterium* 59SB (MN540915.1), *Pantoea eucrina* 85LB (MN541091.1), and *Bacillus aryabhattai* 88LB (MN540958.1), and six fungal strains, *Lasiosphaeriaceae* sp. 17 RF (MN541090.1), *Chaetomium globosum* 37 RF (MN541117.1), *Aspergillus niger* 46SF (MN540962.1), *Peyronellaea* sp. 48SF (MN540968.1), *Talaromyces amestolkiae* 52SF (MN540956.1), and *Alternaria* sp. 63LF (MN541096.1), were isolated from different parts (roots, stems, and leaves) of *Populus tomentosa* (Sehim and Dawwam 2022).

Phytopathogens

Fusarium oxysporum 20RF, *F. solani* 15RF, *C. gloeosporioides* BJ02, and *C. fructicola* were used as the representative phytopathogens. The *Colletotrichum* isolates were kindly provided by the Laboratory of Genetics and Breeding in Forest Trees and Ornamental Plants at Beijing Forestry University, Beijing, China, while *F. oxysporum* 20RF and *F. solani*15RF were isolated from the roots of infected *Populus tomentosa*.

Isolation of *Fusarium* isolates

The infected root tissues of *P. tomentosa* were obtained from the greenhouse of Beijing Forestry University, Beijing, China (40° 0′ N, 116° 20′ E). Root parts were collected and rinsed under running tap water to remove the soil. Then, they were cut into 5–10 mm segments, surface sterilized using 1% sodium hypochlorite solution for 1–2 min, rinsed thrice using sterile distilled water, and dried on a sterile filter paper. The sterilized tissue segments were placed on potato dextrose agar (PDA) plates supplemented with 50 mg/l streptomycin sulfate. The plates were incubated at 28 °C for 5 days for fungal mycelial growth. Mycelial disks were excised from the growing point, subcultured on PDA slants, incubated at 28 °C for 5 days, and stored at 4 °C until further use (Yamauchi et al. 2004).

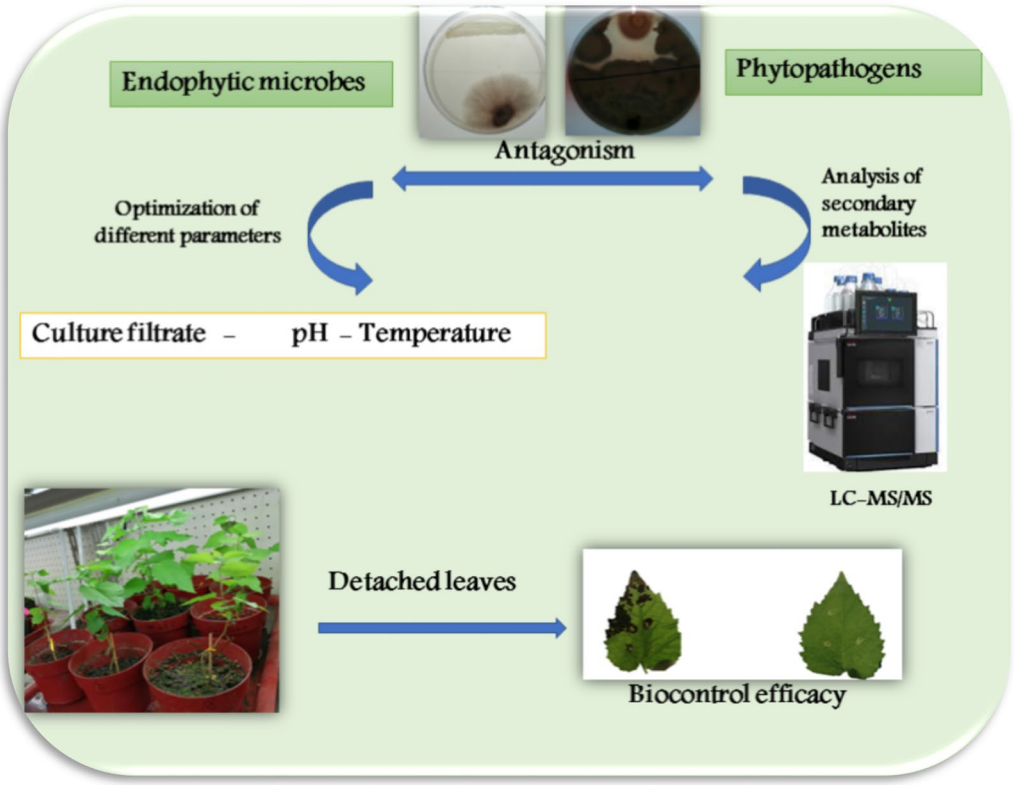

Fig. 1 Graphical abstract for the biocontrol efficacy of endophytic microbes under investigation

Molecular identification

The fungal DNA was extracted using the EZgene™ Fungal DNA Miniprep kit, according to the manufacturer's instructions. The fungal ITS region was amplified using forward ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and reverse ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers (White et al. 1990). The PCR cycle included preheating for 5 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, annealing at 55 °C for 40 s, extension at 72 °C for 1 min, and a final extension of 72 °C for 10 min. The size of the PCR products was checked using 1% agarose gel and sequenced on the ABI3730XL DNA Analyzer at the Beijing Ruibio Biotech, Co., Ltd.

growth-inhibiting fungal pathogens was performed. The biocontrol strains were tested preliminarily using the dual culture method (Huang et al. 2015). The endophytic bacteria and fungi were placed at one end of the PDA plate ($d = 90$ mm), while the pathogenic fungi were placed on the other end, approximately 3 cm away. The plates were cultured at 28 °C for seven days. The control plates were inoculated only with the pathogens. Each treatment was performed in triplicate. The pathogens and control colony diameters were measured after incubation (Boukaew and Prasertsan 2014). The inhibition rate was determined using the formula below:

$$\text{Inhibition growth radial (\%)} = \frac{\text{Radial growth of control} - \text{Radial growth of treatment}}{\text{Radial growth of control}} \times 100.$$

In vitro assessment of endophytic microbes against several fungal phytopathogens

An antagonistic test to study the effect of endophytic bacterial and fungal isolates against several

B. velezensis 33RB and *A. niger 46SF* against *C. gloeosporioides* and *F. oxysporum* were selected for further experiments as they displayed the highest growth inhibitory activity.

Optimizing cultural filtrate

Cultural filtrate method according to the method described by Dennis and Webster (1971) was performed. The bacterial and fungal isolates were inoculated separately for 48 h and 14 days, respectively, in liquid PD medium at 28 °C and 120 rpm. After incubation, the fermentation broth was centrifuged at 10,000 rpm at room temperature for 2 min to remove the remaining bacterial cells and mycelial fragments from the supernatant. The supernatant was transferred into fresh tubes and then filtered twice using sterilized filter paper, followed by 0.45 µm pore membrane filters. Four volumes (50, 25, 12.5, and 6.25 ml) of bacterial and fungal supernatants were mixed thoroughly with molten PDA medium to reach a final concentration of 100% (v/v). The filtrates were poured into Petri plates, and after solidification, a 5-mm disk of test pathogens was placed at the center of the plates. All the treatments were conducted in triplicates and incubated at 28 °C for seven days. The growth inhibition percentage was measured using the equation mentioned above.

Effect of cultural filtrate pH on the growth inhibition

The selected endophytic bacterial and fungal isolates were separately grown on PDA and distributed into media adjusted to five different pH values (3, 5, 7, 9, and 11). Sterilized cultural filtrate (12.5 ml) was added to an appropriate amount of molten PDA till a final concentration of 100% (v/v). After pouring into Petri dishes, it was inoculated with 5-mm mycelial disks containing a 5-day-old colony of test pathogens. After incubation at 28 °C for seven days, the colony diameter of the test pathogens was measured to assess the growth inhibition rate compared to control. This test was done in triplicate.

Effect of cultural filtrate temperature on the growth inhibition

To investigate the effect of temperature on fungal growth, the sterilized cultural filtrate was exposed to 4, 28, 37, 60, 80, and 100 °C for 30 min. After cooling the samples at room temperature, they were tested using the same procedure as the pH test. The formula for relative inhibition rate was described above.

Evaluation of biocontrol efficiency using detached leaves

To evaluate the biocontrol efficacy of bacterial and fungal isolates, sterilized leaves of *P. tomentosa* without any apparent disease symptoms were added into Petri dishes containing a wet filter paper. The petioles of the leaves were wrapped with sterilized pledges and soaked in sterile distilled water for 5 s. These leaves were inoculated with 10 µl of spore suspension with the different test pathogens (1×10^6 spore/ml). After 24 h, all the punctured locations on the leaves were injected with the same volume of biocontrol bacterial and fungal isolates. The positive control was treated with the pathogen only. The leaves were kept at 25 °C in the dark. After 14 days, the lesion diameters were observed and compared.

Identification of antimicrobial secondary metabolites using liquid chromatography–mass spectroscopy (LC–MS spectroscopy)

Metabolites preparation and extraction

The metabolites were extracted with partitioning with ethyl acetate according to the instructions by Novogene, Beijing, China. Then, the bacterial and fungal supernatants (100 µl) were combined with 400 µl of 80% methanol and 0.1% formic acid and vortexed. After centrifuging at 15,000 g, the samples were incubated at 4 °C for 5 min on ice. Then, some of the supernatants were diluted to a final concentration with 53% methanol using LC–MS grade water. The samples were subsequently transferred to fresh Eppendorf tubes and centrifuged at 15,000 g at 4 °C for 10 min. Finally, the supernatant was injected into the LC–MS/MS system for analysis.

UHPLC–MS/MS analysis

LC–MS/MS analyses were conducted using a Vanquish UHPLC system (Thermo Fisher), Beijing, China, coupled with an Orbitrap Q Exactive series mass spectrometer (Thermo Fisher). The samples were injected onto a Hyperil Gold column (100×2.1 mm, 1.9 µm) using a 16-min linear gradient at a flow rate of 0.2 ml/min. The eluents for the positive polarity mode were eluent A (0.1% formic acid in water) and eluent B (methanol). The eluents for the negative polarity mode were eluent A (5 mM ammonium acetate, pH 9) and eluent B (methanol). The solvent gradient was set as follows: 2% B, 1.5 min; 2–100% B, 12 min; 100% B, 14 min; 100–2% B, 14.1 min; 2% B, 17 min. The Q Exactive series mass spectrometer was operated in positive/negative polarity mode with a spray voltage of 3.2 kV, capillary temperature of 320 °C, sheath gas flow rate of 35 arb, and aux gas flow rate of 10 arb. KEGG database (http://www.genome.jp/kegg/) and LIPID MAPS database (http://www.lipidmaps.org/) were used to annotate the metabolites.

Data analysis

The data were statistically analyzed using R software (version 3.6.1) (Team 2016).

Results

Isolation and molecular identification of *Fusarium* isolates

Fusarium oxysporum 20RF and *F. solani* 15RF were isolated from the infected *P. tomentosa* root tissues and confirmed using molecular identification. They were submitted in the GenBank database under accession numbers (ON557730 and ON557719), respectively.

Table 1 Effect of endophytic microbes on growth inhibition of plant pathogenic fungi

Microbial endophytes	Relative mycelial growth inhibition of pathogenic fungi (%)			
	C. gloeosporioides	*F. oxysporum*	*C. fructicola*	*F. Solani*
Enterobacter sp. 24RB	0.0	29.7	25.1	28.5
Bacillus velezensis 33RB	61.2	49.4	30.25	29.1
Bacillus megaterium 59SB	23.7	29.8	19.4	28.9
Pantoea sp. 85LB	24.9	0.0	14.06	18.8
Bacillus aryabhattai 88LB	0.0	22.4	0.0	23.5
Lasiosphaeriaceae sp. 17 RF	5.55	10.2	24.19	21.73
Chaetomium globosum 37 RF	19.44	4.08	1.6	20.28
A. niger 46 SF	75.51	70.83	58.06	60.70
Peyronellaea sp. 48 SF	18.05	6.12	3.2	22.24
Talaromyces amestolkiae 52 SF	52.77	48.97	39.45	26.08
Alternaria sp. 63LF	6.96	0.0	1.6	4.34

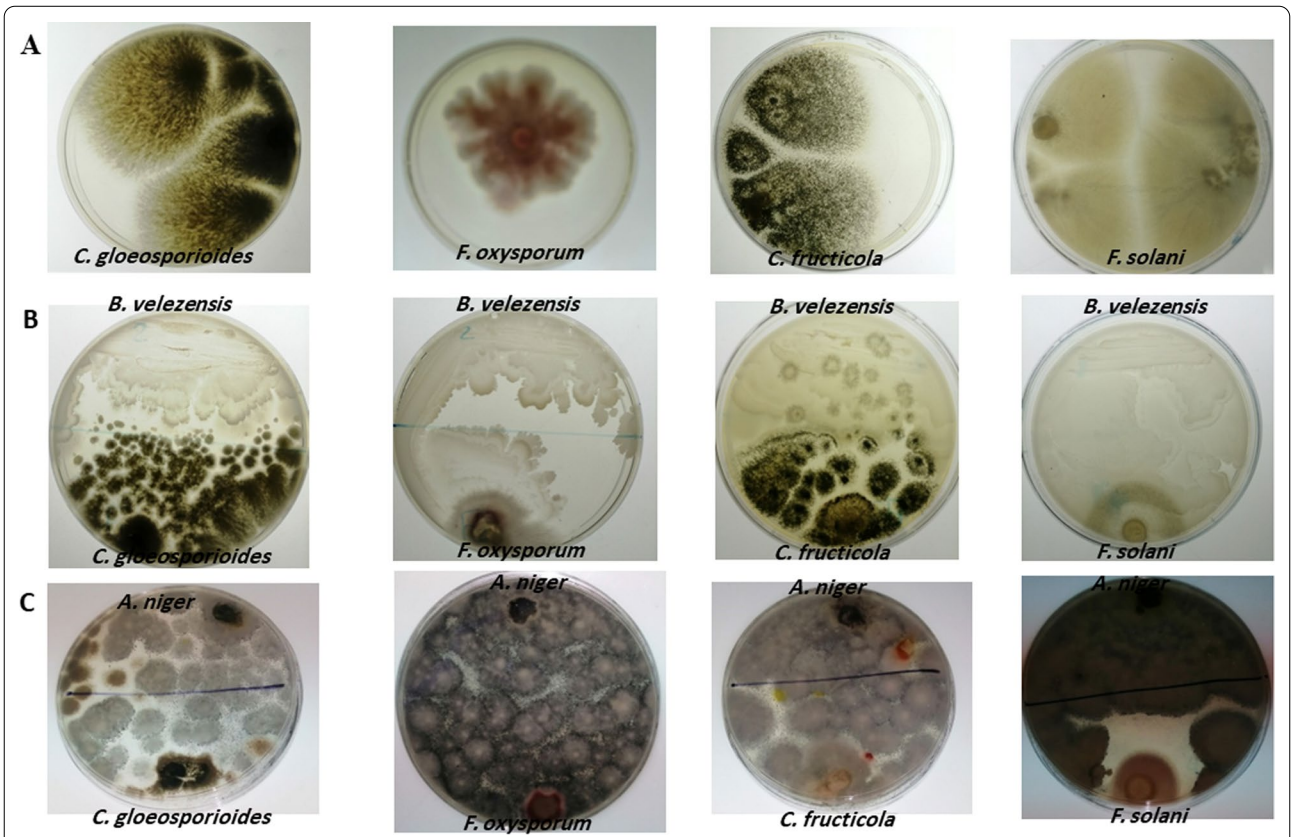

Fig. 2 Antimicrobial effect of *Bacillus velezensis*33RB and *Aspergillus niger* 46SF against *Colletotrichum gloeosporioides* BJ02, *Fusarium oxysporum* 20RF, *C. fructicola, and F. solani* 15RF

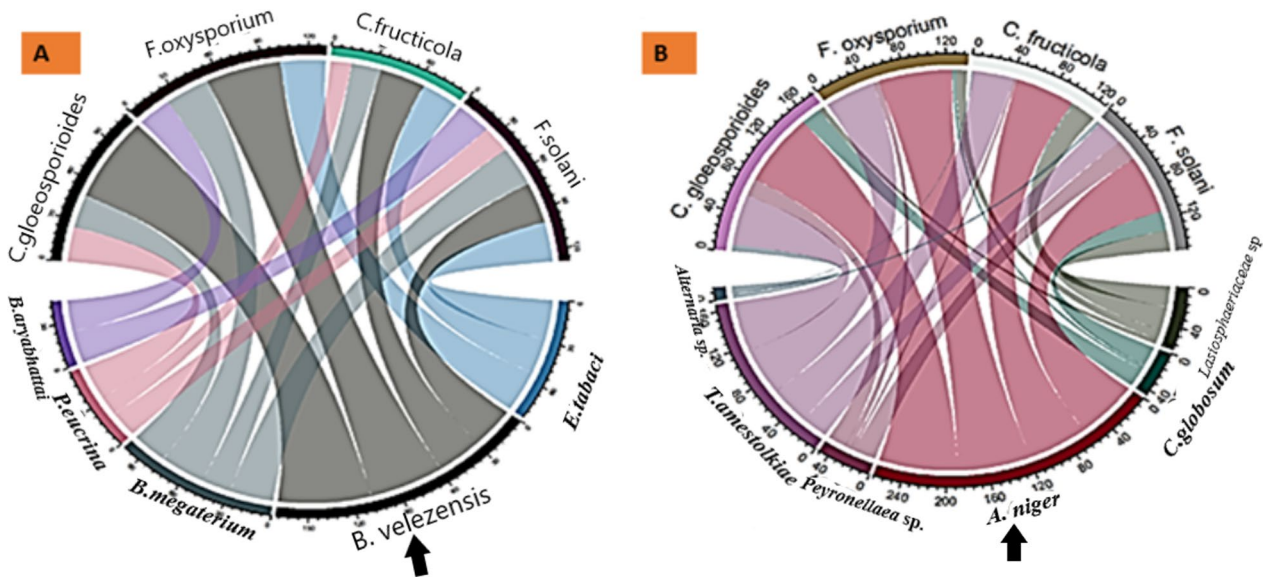

Fig. 3 Chord diagrams represent antifungal activities between phytopathogens and the most efficient bacterial and fungal isolates

Antifungal activities against phytopathogens

Antifungal activity of several plant growth-promoting microbes against phytopathogens such as *F. oxysporum* 20RF, *C. gloeosporioides*BJ02, *F. solani*15RF, and *C. fructicola* using the dual culture method was assessed. The growth inhibitory effect of the endophytes against the pathogenic fungi is shown in Table 1 and Figs. 2, 3. *B. velezensis*33RB displayed the highest inhibitory activity toward *C. gloeosporioides*BJ02 and *F. oxysporum*20RF (61.2 and 49.4%, respectively). However, *A.*

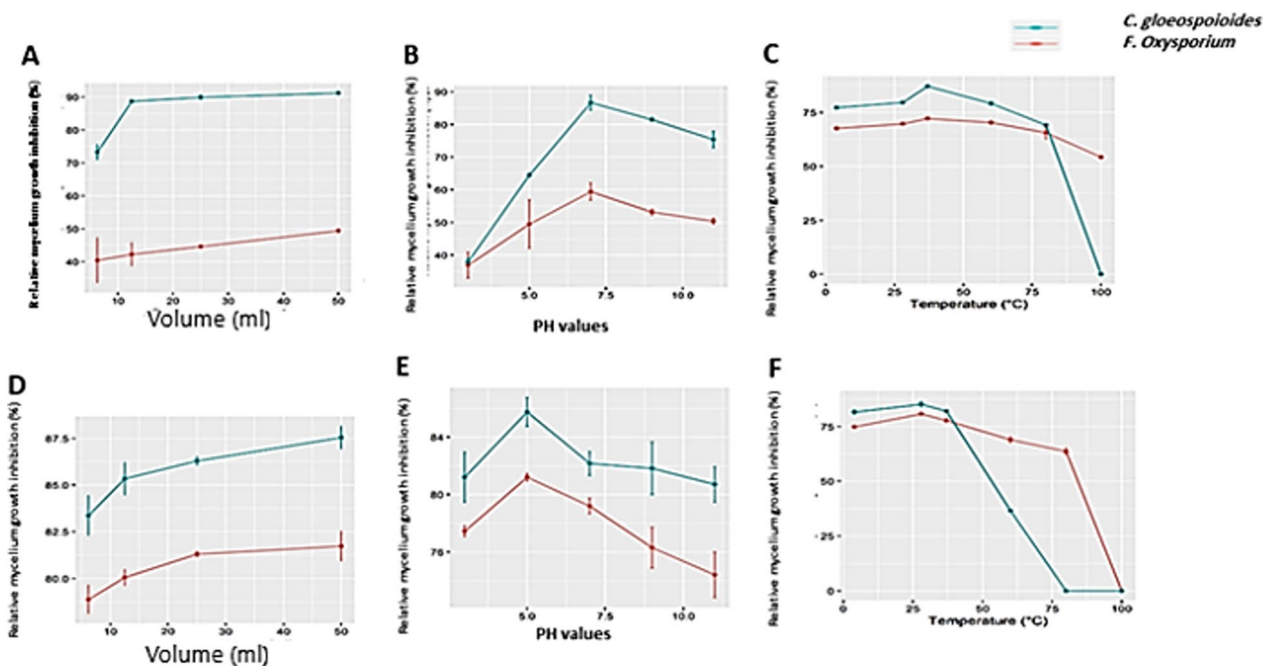

Fig. 4 Different key parameters affect the growth of phytopathogens. **A** Different cultural filtrate volumes, **B** pH values, and **C** different temperatures of *Bacillus velezensis*33RB suspension affect the growth of phytopathogens. **D**–**F** Different cultural filtrate concentrations, pH values, and different temperatures of *Aspergillus niger* 46SF suspension affect the growth of phytopathogens

*niger*46SF showed the strongest antagonistic effect against all the tested pathogenic fungi compared to the other selected isolates. The maximum inhibitory activities (75.51 and 70.83%) were exhibited against *C. gloeosporioides*BJ02 and *F. oxysporum* 20RF, respectively.

Parameters affecting growth inhibition

Cultural filtrate

The sterilized *B. velezensis*33RB and *A. niger*46SF cultural filtrate significantly inhibited the growth of *C. gloeosporioides*BJ02 and *F. oxysporum*20RF (Fig. 4A, D). With the increase in the volume of the sterilized cultural filtrate, the inhibitory activity increased. At minimum volume (6.25 ml), the relative inhibitory rates exhibited by *B. velezensis*33RB and *A. niger*46SF against *C. gloeosporioides* BJ02 and *F. oxysporum* 20RF were (73.3 and 40.4%) and (83.3 and 78.8%), respectively. Clearly, *B. velezensis*33RB and *A. niger*46SF strongly antagonized these phytopathogens.

Effect of pH

The sterilized cultural filtrate was tested under different pH values. Under strongly acidic, neutral, and weakly alkaline conditions, the effect of the sterilized *B. velezensis* 33RB and *A. niger* 46 SF cultural filtrates against *C. gloeosporioides*BJ02 and *F. oxysporum*20RF were significantly different. At neutral pH, *B. velezensis* 33RB showed the highest inhibitions against *C. gloeosporioides* BJ02 and *F. oxysporum*20RF (86.6 and 59.4%, respectively). The maximum growth inhibitions were exhibited by *A. niger*46 SF against *C. gloeosporioides* BJ02 and *F. oxysporum* 20RF (85.7 and 81.2%, respectively) at pH 5. The inhibitory capacity decreased in strongly acidic and weakly alkaline conditions (Fig. 4B, E).

Effect of temperature

The *B. velezensis*33RB filtrate was thermally stable, as shown by the increase in the inhibition rate to over 65% when the filtrate temperature was increased from 4 to

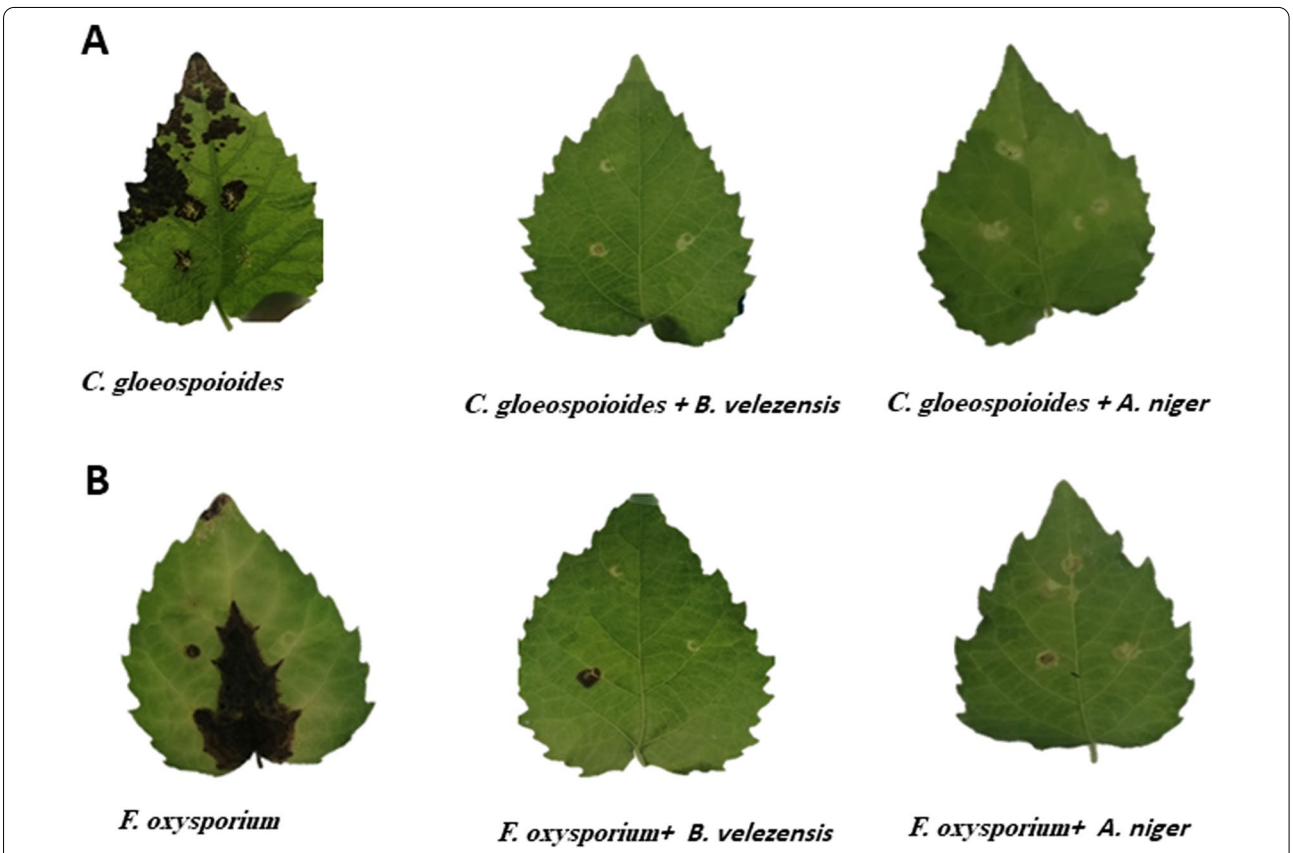

Fig. 5 Photo of biocontrol efficacy of *Bacillus velezensis* 33RB and *Aspergillus niger* 46 SF against *Colletotrichum gloeosporioides* BJ02 and *Fusarium oxysporum*20RF. **A** Spore suspension of C. *gloeosporioides* BJ02 was applied firstly then sterilized cultural filtrate of *B. velezensis* 33RB, *A. niger* 46SF was applied to the same place. **B** Spore suspension of *F. oxysporium* 20RF was applied firstly then sterilized cultural filtrate of *B. velezensis* 33RB, *A. niger* 46SF was applied to the same place

80 °C. The inhibition rate declined at temperatures above 60 °C (Fig. 4C). *B. velezensis*33RB showed the highest inhibitions at 37 °C (72.2 and 87.2%) against *F. oxysporum* 20RF and *C. gloeosporioides* BJ02, respectively. While *A. niger* 46SF displayed the highest inhibition (85.18 and 80.78%) against *C. gloeosporioides* BJ02 and *F. oxysporum* 20RF, respectively, at 28 °C, its inhibitory activity decreased at temperatures above 28 °C (Fig. 4F).

Biocontrol efficiency on isolated leaves

As shown in Fig. 5, the disease control efficacy of *B. velezensis* 33RB and *A. niger*46 SF against anthracnose and fusarium wilt diseases**.** The lesions on the leaves treated with *B. velezensis* 33RB and *A. niger*46SF spore suspensions were smaller than that in control leaves, indicating that *B. velezensis* 33RB and *A. niger*46SF displayed significant preventive effects.

LC–MS spectroscopy analysis of secondary metabolites in the culture extract of B. velezensis 33RB and A. niger 46SF

The presence of different antimicrobial metabolites using LC–MS spectroscopy was investigated. Thirty-three bioactive metabolites were found in the *B. velezensis*33RB culture extract using the negative ion mode (Additional file 1: Table S1). In the *A. niger*46SF culture extract, 36 metabolites were detected in the negative mode (Additional file 1: Table S2). Data showed that number of organic acids was predominant in the cultural extract for both antagonistic microbes. The most predominant acids were citric, valeric, fumaric, phosphoric, succinic, malic, valeric and tartaric acids. Also, numerous fatty acids such as linoleic, caprylic, nonanoic, decanoic, pentadecanoic, stearic, and myristic acids were detected in both microbial extracts.

The features of the *B. velezensis* 33RB and *A. niger* 46SF metabolites were also evaluated using KEGG pathway analysis (Additional file 1: Fig. S1). This revealed that the metabolites belonged to the amino acid, carbohydrate, energy, lipid, cofactors, vitamins, terpenoids and polyketides, xenobiotics biodegradation, membrane transport, and signal transduction pathways. The lipid map indicated that most metabolites were fatty acids and conjugates [FA01]. Eicosanoids [FA03], bile acids and derivatives [ST04], steroid conjugates [ST05], fatty alcohols [FA05], fatty amides [FA08], and octadecanoids [FA02] are also present in Additional file 1: Fig. S2. Thus, several bioactive secondary metabolites were found in *B. velezensis* 33RB and *A. niger* 46 SF culture filtrates, which might enable their antifungal activity.

Discussion

Plant fungal infections are hard to control and, hence, cause significant economic loss. Therefore, management approaches against plant fungal infection, particularly using biological control, are required (Sellem et al. 2017). A dual culture test as a representative test for screening biological control agents was performed. Endophytes with plant growth-promoting and antibacterial properties are potentially beneficial. Both endophytic microbes (*B. velezensis* 33RB and *A. niger* 46 SF) demonstrated potent biocontrol efficacy suggesting that these isolates can suppress the proliferation of phytopathogens, as they significantly inhibited at least three investigated microbial pathogens. Jinfeng et al. (2017) explored the antibacterial activity of endophytes and observed that their crude filtrate inhibited microbial growth. Our findings implied that bacterial and fungal endophytes could act as biocontrol agents by inhibiting harmful pathogens. Consistently, Huang et al. (2020) observed that out of the 15 bacterial strains, *B. subtilis* ZSH-1 displayed the highest antifungal activity against *C. gloeosporioides* (at a distance of 10 mm from the disk). Additionally, Tiwari et al. (2011) demonstrated that *A. niger* suppressed the growth of *Trigonospila cingulata* and *Stereum hirsutum* by 66.29 and 59.26%, respectively.

The cultural filtrate technique was used to assess the in vitro inhibitory effect of the secondary metabolites against mycopathogenic growth. Previous studies have shown that the *Bacillus* and *Aspergillus* cultural filtrates have several properties. Rong et al. (2019) found that the *B. safensis* B21 cultural filtrate displayed antifungal activity against *Magnaporthe oryzae*, which causes rice blast disease. Also, Li et al. (2018) confirmed that the sterilized *B. tequilensis* GYLH001 cultural filtrate significantly inhibited the growth of *M. oryzae*. Furthermore, Huang et al. (2020) showed that the sterilized *B. subtilis* ZSH-1 cultural filtrate displayed high antifungal activities against seven fungal phytopathogens (*C. gloeosporioides, A. tenuissima, F. oxysporum, C. chrysosperma, Mucor* sp., *B. dothidea,* and *Absidia* sp.) with inhibition rates ranging between 44 and 89.1%. Obtained results showed that the sterilized cultural filtrate could effectively inhibit the investigated pathogens at the lowest volume (6.25 ml). Similarly, Idan et al. (2017) found that a low concentration of secondary metabolites (12.5%) of a 14-day-old *A. niger* culture optimally inhibited *P. oryza* growth.

Effect of different temperature and pH ranges was evaluated to understand the physicochemical parameters for microbial growth. pH variations affected the production of secondary metabolites and antimicrobials, probably because the concentration of hydrogen ions might directly impact the cell behavior or might act indirectly due to the different dissociation levels of various components in the growth medium (Li et al. 2018). Resultantly, pH changes significantly affected the enzyme activities of microorganisms, and the generation, dissociation, and solubility of intermediate compounds (Kumar

et al. 2003). Additionally, the incubation temperature is a physical component that might variably affect the microorganisms' growth and generation of secondary metabolites (Jain et al. 2011).

The present results revealed that *B. velezensis* 33RB exhibited the highest inhibitions against *C. gloeosporioides*BJ02 and *F. oxysporum* 20RF at neutral pH and was also thermally stable. Consistently, Li et al. (2018) found that the sterilized cultural filtrate of *B. tequilensis* GYLH001 showed the strongest inhibition against *M. oryzae* at pH 7 and was thermally stable. The inhibition rate reached 60%, when the filtrate's temperature ranged between 4 and 80 °C. Beyond 60 °C, the inhibition rate decreased with an increase in temperature. The results revealed that *A. niger*46SF exhibited the maximum growth inhibitions against *C. gloeosporioides* BJ02 and *F. oxysporum*20RF at pH 5 and 28 °C. Similarly, Idan et al. (2017) observed that pH 5 was ideal for the production of antimicrobials by *A. niger*.

Poplar anthracnose is mainly treated using fungicidal treatments (Song et al. 2016). Numerous *Bacillus* species have been employed as biocontrol agents against *C. gloeosporioides* (Guerrero-Barajas et al. 2020). Bacterial secondary metabolites are organic, low-molecular-weight chemicals that suppress other microbes (Thomashow 2002). LC–MS analysis showed that the secondary metabolites produced by *B. velezensis* 33RB and *A. niger* 46SF isolates possessed several antimicrobial, herbicidal and insecticidal activities. Numerous bacterial genera, including *Bacillus*, *Agrobacterium*, *Pantoea, Serratia*, *Pseudomonas*, *Streptomyces,* and *Stenotrophomonas,* have been shown to produce antimicrobial metabolites with broad-spectrum activities (Ongena and Jacques 2008). Moreover, *A. niger* is a significant source of secondary metabolites (Al-Shaibani et al. 2013).

The metabolites detected in the present study were mostly organic acids such as fumaric, DL-malic, citric, isobutyric, 2-keto-glutamic, levulinic, succinic, malonic, and aspartic acids. All these acids reduced the pH and suppressed microbial growth (Eklund 1989). The hydrophobic and undissociated forms of the acid caused cell membrane diffusion and detachment from the cell, resulting in the release of hydrogen carbon ions and acidification of the cytoplasm (Piard and Desmazeaud 1991). Russell (1991) found that many types of fatty acids have antifungal and antibacterial characteristics. Several fatty acids, including lauric, linoleic, stearic, oleic, and myristic acids, which have been previously reported to have antibacterial and antifungal properties, were detected (Seidel and Taylor 2004). Hence, the presence of fatty acids might have contributed to the reduction of *F. oxysporum*20RF and *C. gloeosporioides*BJ02 mycelial growth.

Conclusion

The results revealed the antifungal potential of the endophytic isolates *B. velezensis*33RB and *A. niger* 46SF, which showed antagonistic activity against taxonomically diverse fungal pathogens, especially *Colletotrichum* and *Fusarium* genera that infect *Populus tomentosa*. Different antimicrobial metabolites with diverse biological functions were determined using LC–MS spectroscopy. The most detectable compounds included organic acids such as fumaric, DL-malic, isobutyric, and glutamic acids. Also, numerous fatty acids such as lauric, linoleic, oleic, stearic, and myristic acids were determined. In summary, *B. velezensis*33RB and *A. niger* 46SF were promising biological control agents for anthracnose and fusarium wilt diseases management affecting *P. tomentosa*.

Supplementary Information

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Additional file 1. Table S1. Bioactive secondary metabolites obtained from *Bacillus velezensis* 33RB using LC-MS/MS analysis. **Table S2**. Bioactive secondary metabolites extracted from *A. niger* 46SF using LC-MS/MS analysis. **Fig. S1**. KEGG Pathway of different metabolites produced by *B. velenzensis* and *A. niger*. (A&B) KEGG Meta negative and positive Annotation of *B. velenzensis*. (C&D) KEGG Meta negative and positive Annotation of *A. niger*. **Fig. S2**. Lipid maps analysis of bioactive metabolites of *B. velenzensis* and *A. niger* by negative and positive annotation. (A&B) Lipid maps of Meta negative and positive Annotation of *B. velenzensis*. (C&D) Lipid maps of Meta negative and positive Annotation of A. niger.

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Author contributions

GED, AES, conceptualization, and supervision; GED, AES conducted the experiment; GED, AES wrote original draft preparation, GED, AES wrote review and editing. Both authors contributed equally to the work. Both authors have read, reviewed, and agreed to publish the version of the manuscript.

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Availability of data and materials

The sequencing data generated and analyzed in this study are available in the NCBI Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/), Accession Number: ON557719 and ON557730.

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

All the authors have given their consent to publish the submitted manuscript as an "Original paper" in EJBPC.

Competing interests

The authors declare that they have no competing interests.

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