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Identification of Seed and Soil-Borne Fungal Disease Agents in Okra Plants (*Abelmoschus esculentus* L.) and Characterization of Biological Control Possibilities with Antagonist Bacterial Isolates

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ABSTRACT
The main objectives of this study were to (i) identify the seed- and soil-borne fungal pathogens of okra (*Abelmoschus esculentus* L.) grown in the Hatay province of Türkiye, (ii) determine the *in vitro* biocontrol potential of bacterial isolates (BCAs) from healthy okra and closely related plants against common fungal pathogens, and (iii) characterize the antagonistic and plant growth-promoting (PGP) mechanisms involved in pathogen suppression and plant growth. *Rhizoctonia solani, Macrophomina phaseolina, Sclerotinia sclerotiorum*, and *Fusarium oxysporum* were determined as the most common soil and seed-borne disease agents on diseased seeds and plants. A total of 36 different endophytic and epiphytic bacterial isolates were selected and identified using MALDI-TOF MS, and their antagonistic potentials to inhibit mycelial growth of *R. solani, M. phaseolina, S. sclerotiorum*, and *F. oxysporum* were characterized in dual culture tests. Among BCA bacterial isolates, *Bacillus cereus* B1ep, *B. cereus* B2ep, *Pseudomonas aeruginosa* B3ep, *B. cereus* B11ep, and *B. subtilis* B12ep displayed high levels (≥70%) of antagonistic activity against all fungal agents tested. *Enterobacter cloacae* B10ep was identified as the highest siderophore producer, *Microbacterium maritypicum* X5 as the highest protease enzyme producer, *Pseudomonas aeruginosa* B3ep, and different *Bacillus* spp. as isolates producing hydrogen cyanide (HCN) and ammonia at the highest level. The suppression of mycelial growth was suggested to be associated with one or more antagonistic mechanisms. *Enterobacter bugandensis* B7ep was identified as the highest Indole-3 Acetic Acid (IAA) producer, and *B. cereus* P7en was identified as the most efficient phosphate-solubilizing bacterial isolate. In conclusion, BCA isolates belonging to *Bacillus* and *Pseudomonas* spp., which demonstrated strong antagonistic and PGP activities, have the potential to be developed as biopreparations against soil and seed-borne disease agents in okra plants.

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Bamya Bitkisinde (*Abelmoschus esculentus* L.) Tohum ve Toprak Kaynaklı Fungal Hastalık Etmenlerinin Tanılanması ve Antagonist Bakteri İzolatları ile Biyolojik Mücadele Olanaklarının Karakterizasyonu

ÖZET
Bu çalışmanın amaçları, (i) Türkiye'nin Hatay ilinde yetiştirilen hastalıklı bamya (*Abelmoschus esculentus* L.) bitkilerinin tohum ve toprak kaynaklı fungal patojenlerini tanımlamak, (ii) sağlıklı bamya ve yakın akraba bitkilerden elde edilen epifitik ve endofitik biyokontrol etmeni bakteri izolatlarının (BCA) yaygın fungal patojenlere karşı *in vitro* biyokontrol potansiyellerini belirlemek ve (iii) patojen baskılanmasında ve bitki gelişiminde rol oynayan antagonistik ve bitki büyümesini teşvik edici (PGP) özellikleri karakterize etmektir. Hasta bitkilerden ve tohumlarından yapılan izolasyonlar sonucunda en yaygın toprak ve tohum kaynaklı fungal hastalık etmenler *Rhizoctonia solani, Macrophomina phaseolina, Sclerotinia sclerotiorum* ve *Fusarium oxysporum*, olarak belirlenmiştir. Toplam 36 farklı endofitik ve epifitik

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bakteri izolatı seçilmiş, MALDI-TOF MS ile tanımlanmış ve *R. solani, M. phaseolina, S. sclerotiorum* ve *F. oxysporum*'un misel büyümesini engellemedeki antagonistik potansiyelleri ikili kültür testleri ile karakterize edilmiştir. BCA bakteri izolatları arasında *Bacillus cereus* B1ep, *B. cereus* B2ep, *Pseudomonas aeruginosa* B3ep, *B cereus* B11ep ve *B. subtilis* B12ep, test edilen tüm fungal hastalık etmenlerine karşı yüksek düzeyde (≥%70) antagonistik etkinlik göstermiştir. *Enterobacter cloacae* B10ep en yüksek siderofor üreticisi, *Microbacterium maritypicum* X5 en yüksek proteaz enzimi üreticisi, *P. aeruginosa* B3ep ve farklı *Bacillus* spp. ise en yüksek düzeyde hidrojen siyanür (HCN) ve amonyak üreten izolatlar olarak tanımlanmıştır. Misel büyümesinin baskılanmasında bir veya daha fazla antagonistik mekanizma ile ilişkili olduğu tespit edilmiştir. *Enterobacter bugandensis* B7ep en yüksek Indol-3 Asetik Asit (IAA) üreticisi, *B. cereus* P7en ise en etkili fosfor çözen bakteri izolatı olarak tanımlanmıştır. Sonuç olarak, güçlü antagonistik ve PGP aktiviteleri gösteren *Bacillus* ve *Pseudomonas* spp. türlerine ait BCA bakteri izolatları, bamya bitkilerinde toprak ve tohum kaynaklı hastalık etkenlerine karşı biyolojik preparat olarak geliştirilme potansiyeline sahiptir.

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INTRODUCTION

Okra (*Abelmoschus esculentus* L.), a member of the Malvaceae (mallow) family, is one of the most widely cultivated vegetables in tropical and subtropical regions worldwide due to its drought tolerance, affordability, rich vitamin content, and nutritional value. Particularly prominent in East Asian and Indian cuisines, okra is primarily consumed fresh during summer months in Türkiye. In recent years, it has also been made available year-round as a frozen product in supermarkets. Given the high seed count and oil content of the okra plant, research has been conducted not only on its use as a vegetable but also on its potential as an oilseed crop (Ayub et al., 2021).

In 2023, global okra production reached 11,523,291 tons cultivated across 2,949,566 ha (Anonymous, 2021). India dominated production with 7,158,000 tons, followed by Nigeria, Sudan, Mali, Côte d'Ivoire, Pakistan, Iraq, Egypt, and Ghana. In Türkiye (2023), okra was cultivated on 4,379 ha, yielding 29,120 tons (Anonymous, 2023). The top producing provinces were Balıkesir, 734 ha, Amasya, 540 ha, Aydın, 343 ha, İzmir, 335 ha, and Osmaniye, 271 ha. Notably, Hatay ranked 13th, producing 611 tons from 55 ha, demonstrating higher yield efficiency (~11.1 tons/ha) compared to the national average (~6.7 tons/ha).

Globally and in Türkiye, the rising demand for okra has led to an expansion of cultivation areas. However, this growth has been accompanied by significant yield losses due to fungal, bacterial, and viral diseases, as well as pest infestations (Sitara et al., 2020). In major okra-producing regions, particularly in Africa and East Asia, pathogen-induced crop damage has been widely documented. Studies have identified numerous seed-, soil-, and foliar-borne fungal and bacterial pathogens affecting diverse okra varieties across different agroecological zones.

Among disease agents affecting okra, powdery mildew (*Erysiphe cichoracearum* DC. ex-Merat) has been identified as the most prevalent foliar pathogen. A comprehensive survey in Pakistan documented 14 fungal pathogens, including 10 seed-borne species, with the most yield-limiting species being: Damping-off/seedling blight (caused by *Fusarium* spp. (including *F. oxysporum*, *F. moniliforme*), Stem rot (caused by *Macrophomina phaseolina*), Anthracnose (caused by *Colletotrichum dematium*), and Dieback diseases (Anam et al., 2002).

Isolations from seeds of diseased okra plants led to the molecular identification of soil-borne fungal pathogens such as *Alternaria alternata*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium oxysporum*, *F. solani*, *F. moniliforme*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Stemphylium botryosum*, *Penicillium digitatum*, and *Pythium aphanidermatum* (Al-Kassim & Monawar, 2000). Morphological diagnostic studies on disease symptoms observed in okra plants—including wilting, leaf spots, stem canker, and fruit blight—identified the pathogens *Fusarium oxysporum*, *Curvularia lunata*, *Fusarium chlamydosporum*, and *Choanephora cucurbitarum*.

Globally, various pathogens, such as *Phytophthora nicotianae* in Iraq (Mathny, 2013), *Verticillium dahliae* in

Northern China (Zhai et al., 2019), *Cercospora malayensis* and *Podosphaera xanthii* in South Korea (Choi et al., 2018; Ju et al., 2020), *Podosphaera xanthii* (Fan et al., 2019), *Alternaria alternata* (Chai et al., 2019), *Colletotrichum gloeosporioides* (Shi et al., 2019), *Choanephora cucurbitarum* (Liu et al., 2019), *Pythium aphanidermatum* (Qu et al., 2021), *Cercospora cf. flagellaris* in China (Chai et al., 2021), *Sclerotium rolfsii* in Côte d'Ivoire (Koné et al., 2010), and *Colletotrichum plurivorum* in Brazil (Batista et al., 2020), have been reported in okra cultivation across different climates and regions.

Based on the literature review, no studies have been conducted to determine the prevalence of soil- or seed-borne fungal or bacterial diseases affecting okra cultivation areas in Türkiye (Farr & Rossman, 2024). However, the presence of powdery mildew caused by *Podosphaera xanthii* (Soylu et al., 2023) and Verticillium wilt caused by *Verticillium dahliae* (Tok et al., 2020) has been reported in okra plants grown in Türkiye.

The main alternative control strategies to chemical methods against plant pest and diseases include use of plant extract and essential oils, disinfectants, physical treatments such as hot water, UV radiation, microwave applications, chemical, abiotic, and biotic elicitors (e.g., salicylic acid, BTH, BABA, INA), biological control using fungal and bacterial isolates (Baysal et al., 2003; Soylu et al., 2010; Sertkaya et al., 2010; Kaya et al., 2018; Bozkurt et al., 2020; Şahin et al., 2021; Türkmen et al., 2022; Atay & Soylu, 2022; Asil et al., 2022; Soylu et al., 2022; Kara et al., 2023; Kara et al., 2024). Among alternative plant disease management strategies, the use of biopreparations containing biological control agents (BCAs) has become a primary approach in recent years. Due to the adverse effects of chemical pesticides, particularly in developed countries, research on sustainable disease management using BCAs has intensified (Cook, 1993; Guo et al., 2004; Huang et al., 2005). Biological control using antagonistic microorganisms (BCAs) has gained prominence as a sustainable alternative to chemical pesticides, particularly under escalating challenges such as climate change and the rise of resistant plant pathogens. Recent studies highlight the adaptive potential of BCAs in mitigating abiotic and biotic stresses while promoting ecosystem resilience (Compant et al., 2025). BCAs like *Trichoderma spp.* and Bacillus spp, *Pseudomonas* spp exhibit thermo-tolerance and enhance host plant resilience under drought and heat stress (Makhlouf et al., 2023). For instance, *Bacillus subtilis* strains modulate plant ethylene pathways, improving stress tolerance while suppressing soil-borne pathogens (Ali et al., 2024). Overuse of agrochemicals has led to pesticide-resistant pathogen strains (e.g., *Fusarium oxysporum*, *Botrytis cinerea*). BCAs employ multifaceted mechanisms—antibiosis, competition, and induced systemic resistance (ISR)—to overcome resistance. For example, *Streptomyces spp.* produces secondary metabolites effective against resistant *Xanthomonas* strains (Palaniyandi et al., 2013). Meta-analyses confirm that microbial consortia reduce resistance evolution by 50% compared to single-mode chemical treatments (Parnell et al., 2016). BCAs align with circular economy principles by leveraging native microbial communities, reducing input dependency. Field trials demonstrate that BCA-integrated systems increase yield by 15–30% in climate-vulnerable regions (Sena et al., 2024). Policy frameworks like the EU's Farm-to-Fork Strategy now prioritize BCAs to meet 2030 pesticide reduction targets (EU Directive 2023/341, Anonymous, 2020).

Comprehensive literature review revealed a limited number of studies investigating alternative control methods against seed- and soil-borne pathogens affecting okra plants, as opposed to chemical treatments (Ehteshamul-Haque et al., 1990; Anam et al., 2002; Okwu et al., 2007; Dawar et al., 2008; Channa et al., 2008; Nwangburuka et al., 2012; Afzal et al., 2013; Aloui et al., 2017; Rama et al., 2017; Grace et al., 2019; Rai et al., 2019; Hossain et al., 2021; Jabeen et al., 2021; Singh et al., 2021; Urooj et al., 2021).

In this study, (i) isolation and identification of soil and seed borne fungal diseases in okra plants grown in different districts of Hatay province of Türkiye, (ii) isolation and identification of endophytic and epiphytic biocontrol agent (BCA) bacterial isolates from different parts of healthy okra and closely related plants; (iii) *in vitro* biocontrol activities of BCA isolates on mycelial growth of common soil and seed borne fungal disease agents in okra plants; (iv) characterization of possible mechanisms of action of BCA isolates involved in inhibition of mycelial growth of fungal agents and plant growth promotion (PGP) were aimed.

MATERIAL and METHODS

Pathogen isolation and identification

During the 2021 growing season, surveys were conducted in okra (*Abelmoschus esculentus*) cultivation areas across multiple districts of Hatay province (Antakya, Kumlu, Kırıkhan, Reyhanlı, Erzin, and Dörtyol). Plants exhibiting disease symptoms were collected and subjected to isolation procedures. Based on morphological characterization, four fungal isolates were identified and designated as follows: *Macrophomina phaseolina* (GAB-Mp12), *Fusarium oxysporum* (GAB-Fox7), *Sclerotinia sclerotiorum* (GAB-Ss23), *Rhizoctonia solani* (GAB-Rs5). These isolates served as the primary fungal pathogens for subsequent experiments in this study.

Isolation and identification of putative antagonistic bacterial isolates

Endophytic and epiphytic bacterial isolates with potential biocontrol activity against fungal pathogens were isolated from various plant tissues (roots, stems, and fruits) of healthy okra (*Abelmoschus esculentus*) plants growing in the same fields where diseased samples and also closely related host plants including cotton (*Gossypium hirsutum*), common mallow (*Malva sylvestris*), and cocklebur (*Xanthium strumarium*) according to method described by Aktan & Soylu, (2020).

Putative bacterial isolates exhibiting different morphological characteristics were subjected to Hypersensitive response (HR) tests on tobacco (*Nicotiana tabacum*) leaves and soft rot assays on potato (*Solanum tuberosum*) tubers (Lelliot & Stead, 1987). Only isolates showing negative results in both HR and soft rot tests were selected for further bioactivity studies. The taxonomic identification of selected bacterial isolates was performed using MALDI-TOF mass spectrometry (Bruker Biotyper, Bruker Daltonics GmbH, Bremen, Germany) according to method described by Aktan & Soylu, (2020). MALDI-TOF MS analysis was performed using the Microflex LT system (Bruker Daltonics GmbH, Bremen, Germany). The recorded protein spectra were compared with the reference spectra in the BIOTYPERTM 1.1 software database. Confidence levels of identification based on the scores: 2.30-3.00: Very reliable identification at species level; 2.00-2.299: Reliable identification to genus level, probable species level; 1.70-1.999: Reliable identification to genus level, possible identification to species level; and <1.70: Unreliable identification. All samples were processed in duplicate. Regular calibration of the MS instrument according to the manufacturer's protocols.

In vitro assessment of antagonistic bacterial isolates on mycelial growth inhibition of fungal pathogens

The antagonistic effects of identified bacterial isolates against fungal pathogens were assessed using dual-culture assays on PDA+TSA (1:1, v/v) medium in 90 mm diameter Petri plates (Gümüş & Soylu, 2024). Bacterial isolates were streaked 2 cm from the upper edge of agar plates and pre-incubated at 25°C for 2 days. After pre-incubation, 6-mm mycelial disks from 5-day-old fungal cultures were placed 4 cm below the bacterial streak. Control plates received only fungal inoculum without bacterial isolates. Sealed plates were incubated at 25°C for 5-7 days.

When control plates showed complete fungal coverage, inhibition percentages were calculated using Abbott's formula:

$Inhibition\ (\%) = \frac{\mathrm{CFG-TFG}}{\mathrm{TFG}}\ x\ 100$, where C_{FG} = Fungal Growth (mm) in Control Petri dishes, and T_{FG}= Fungal Growth (mm) in dual-culture plates.

For each bacterium-fungus combination, measurements were conducted in three separate Petri plates, and the entire experiment was repeated twice to ensure reproducibility of results.

Characterization of biocontrol and plant growth-promoting mechanisms in putative bacterial isolates

The potential mechanisms underlying the biocontrol efficacy (siderofor, protease, hydrogen cyanide (HCN) and ammonia productions) and plant growth promotion (phosphate solubilisation and Indole-3 Acetic Acid (IAA) production) of selected bacterial isolates were evaluated through specific biochemical assays.

Protease activity was assessed using 2% skim milk agar containing Luria Broth Agar plates, with zones of casein hydrolysis indicating protease production (Perneel et al., (2007). Siderophore production was detected on chrome azurol S (CAS) agar plates, with orange halos indicating iron-chelating activity (Schwyn & Neilands, 1987). HCN production was evaluated using nutrient agar supplemented with glycine, with color change in filter paper indicating HCN synthesis (Castric, 1977). Ammonia production was tested in peptone water, with Nessler's reagent detecting ammonia formation (Cappuccino & Sherman, 2013)

Phosphate solubilisation was screened on Pikovskaya's agar containing insoluble tricalcium phosphate, with clear zones indicating solubilization activity (Kumar et al., 2012). IAA production was quantified in tryptophan-supplemented medium using Salkowski reagent, with pink coloration indicating auxin production (Glickman & Dessaux, 1995)

All assays were performed in triplicate under controlled conditions (28±1°C). Quantitative measurements were taken after specified incubation periods (24-72 h depending on assay). Results were documented through both visual observation and spectrophotometric quantification when applicable. The biocontrol and plant growth-promoting potential of each bacterial isolate for siderophore, protease and phosphate solubilization were quantitatively evaluated through the calculation of specific activity indices. Activity indices were calculated according to Vazquez et al. (2000), with final values representing the mean of six measurements (3 plates × 2 trials) for each isolate. The indices provide a normalized measure of bacterial activity, allowing for direct comparison between isolates independent of colony size variations. IAA concentration was measured at 535 nm wavelength using a UV-Vis spectrophotometer (PerkinElmer Lambda 25, USA). Methodology followed established protocols (Patten & Glick, 2002). Bacterial supernatants were reacted with Salkowski reagent (1:2 v/v) for 30 min in darkness. Standard curve prepared using pure IAA (Merck, Darmstadt, Germany) in the range of 0-100 μg mL^{-1}.

Absorbance values of samples were compared against the standard curve. Results expressed in µg mL^{-1} (ppm) of IAA produced. Triplicate measurements were done for each bacterial isolate

This comprehensive approach enables systematic evaluation of multiple plant-beneficial traits, providing mechanistic insights into the observed biocontrol and growth promotion effects. The selected methods represent well-established, standardized protocols in microbial functional characterization studies.

Statistical analysis of *in vitro* experiments

All *in vitro* experiments were conducted using a completely randomized design (CRD), with data subjected to rigorous statistical analysis. Raw inhibition data were analyzed using SPSS Statistics 17.0. One-way ANOVA was performed to assess treatment effects. Post-hoc comparisons were done via Tukey's HSD Test, significance threshold was set at $P \leq 0.01$.

RESULTS and DISCUSSIONS

Isolation and identification of pathogens from okra root and crown, stem, and seed tissues

During surveys conducted in 17 different okra fields across various districts of Hatay province between September and November 2021, a total of 84 plant samples exhibiting disease symptoms were collected. Isolations were performed on appropriate culture media from the roots and root collar rot of plants showing typical wilting symptoms, darkened vascular bundles in stems, and discolored seeds within mature fruits left for seed production (Figure 1). Based on morphological studies of colony growth, sporulation, and mycelial structures of the fungi obtained from the isolations, the most prevalent soil-borne fungal pathogens were identified as follows: *Rhizoctonia solani* (Sneh et al., 1991) from 21 different plant samples, *Macrophomina phaseolina* (Dhingra & Sinclair, 1973) from 26 plant samples, *Sclerotinia sclerotiorum* (Kohn, 1979) from 14 different plants, *Fusarium oxysporum* (Leslie & Summerell, 2006) from 30 different plant samples, and *Verticillium dahliae* (Pegg & Brady, 2002) from 12 different plant samples.

Isolation studies from discolored seeds of symptomatic mature fruits yielded fungal isolates including *Fusarium oxysporum* from 5 different seed samples, *Macrophomina phaseolina* from 6 samples, *Alternaria* spp. from 19 samples, *Aspergillus* spp. from 18 samples, *Rhizopus* spp. from 14 samples, and *Penicillium* spp. from 23 samples (Figure 2). Among these isolates, potential widespread soil and seedborne pathogens specifically *M. phaseolina* (GAB-Mp12), *F. oxysporum* (GAB-Fox7), *S. sclerotiorum* (GAB-Ss23), and *R. solani* (GAB-Rs5) - were purified for use in pathogenicity tests and *in vitro* bioefficacy studies (Figure 3A-D). Based on survey results, pure isolates of prevalent soil and seedborne pathogens *M. phaseolina* (GAB-Mp12), *F. oxysporum* (GAB-Fox7), *S. sclerotiorum* (GAB-Ss23), and *R. solani* (GAB-Rs5) (Figure 3A-D) were re-inoculated into the root zones of healthy okra seedlings. Disease development was evaluated four weeks post-inoculation based on symptoms including root and crown thinning, vascular discoloration, and necrosis in infected plants (Figure 3E). The pathogens were re-isolated from symptomatic tissues at inoculation sites and compared with original isolates. Re-isolates matching the original strains were used in all subsequent trials.

These pathogens have been frequently reported in major okra-producing countries worldwide, including Nigeria, Pakistan, Bangladesh, India, and China (Ehteshamul-Haque et al., 1996; Oluma & Nwankiti, 2000; Sultana et al., 2019; Li et al., 2015; Prova et al., 2017). In Türkiye, apart from *Verticillium dahliae* (Tok et al., 2020), no studies have identified diseases and their causative agents in cultivated okra plants. To the best of our knowledge, this study is the first report of these fungal pathogens (excluding *V. dahliae*) on okra plants in Türkiye.

Isolation and identification of putative epiphytic and endophytic bacterial isolates

Putative epiphytic and endophytic bacterial isolates were obtained from both below-ground (roots, root crowns, and rhizosphere soil) and above-ground (stems near the crown, branches, shoots, leaves, and fruits) tissues of healthy okra plants and related species growing in fields where pathogens had been previously isolated. From these isolations, a total of 71 candidate bacterial isolates were obtained, comprising 28 endophytic (39.4%) and 43 epiphytic (60.6%) strains with distinct morphological characteristics. Each isolate was coded to represent its geographic origin, host plant part, and isolation plate.

Prior to bio efficacy studies, all candidate isolates were screened through hypersensitive response (HR) tests on tobacco leaves and soft rot assays on potato slices. Identification of the 51 isolates (35 epiphytic and 16 endophytic), that tested negative in these pathogenicity screens, was performed using MALDI-TOF MS. Following identification, 36 well-characterized isolates were selected for subsequent *in vitro* bioefficacy studies.

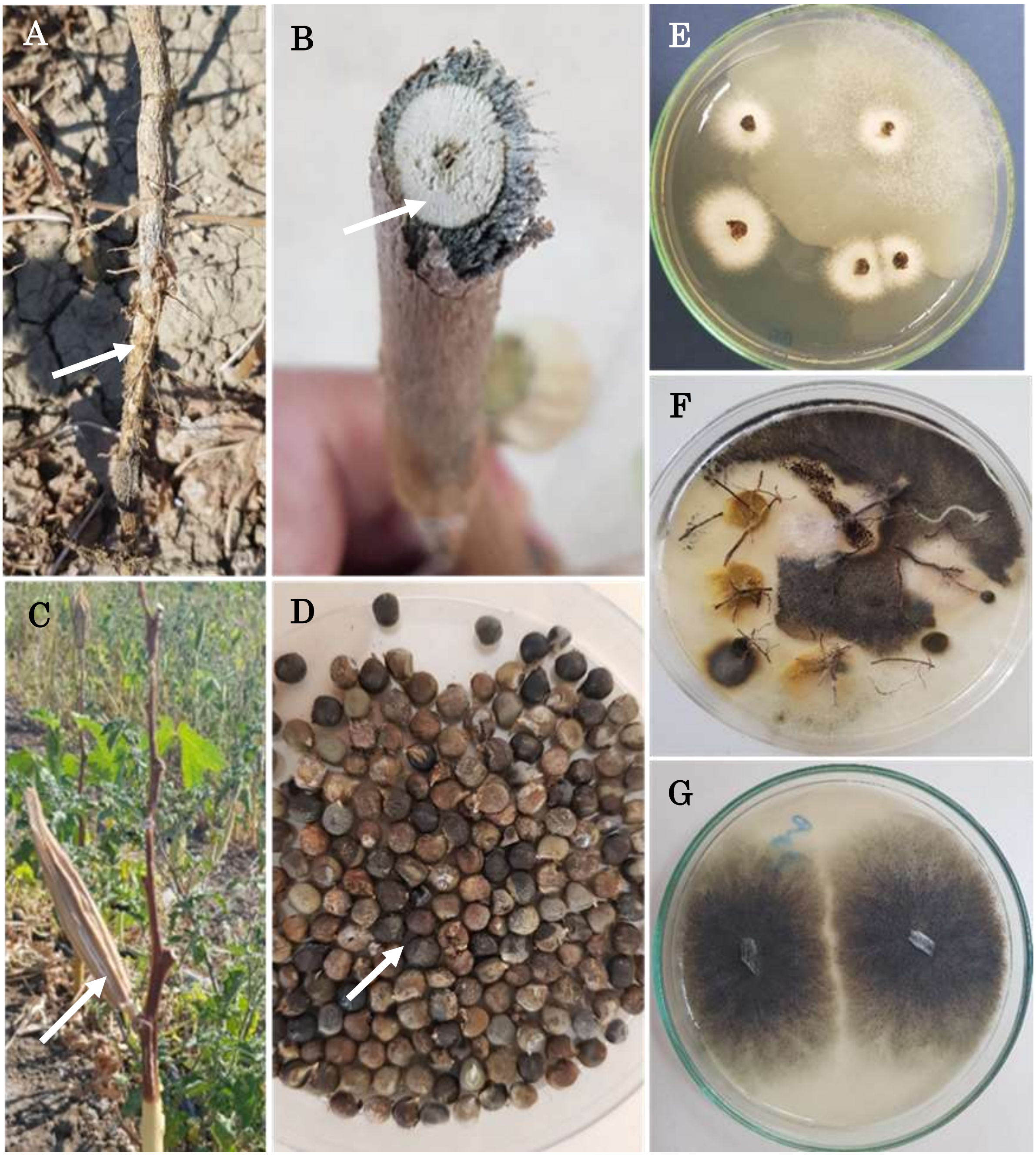

Figure 1. Disease symptoms (arrows) and fungal cultures isolated from different tissues of okra plants (*Abelmoschus esculentus*) across Hatay province. (A-B) Stem lesions, (C) Fruit decay, (D) Discolored seeds, (E-G) Corresponding fungal cultures isolated from symptomatic tissues.

Şekil 1. Hatay ilinde bamya bitkilerinin (Abelmoschus esculentus) farklı dokularından izole edilen hastalık belirtileri (ok) ve fungal kültürleri. (A-B) Gövde lezyonları, (C) Meyve çürümesi, (D) Renk değişikliği gösteren tohumlar, (E-G) Belirtili dokulardan izole edilen karşılık gelen fungal kültürleri.

MALDI-TOF MS identification revealed that the bacterial isolates belonged to 19 distinct species. At the genus level, *Bacillus* spp. was the most prevalent, represented by 25 isolates. This was followed by *Enterobacter* spp. (4 isolates), *Pseudomonas* spp. (2 isolates), *Microbacterium* spp., *Pantoea* spp., *Paenibacillus* spp., *Lysinibacillus* spp., and *Rhizobium* spp. (1 isolate each). Consistent with previous studies, epiphytic and endophytic bacterial isolates belonging to various α, β, and γ Proteobacteria phyla, as well as Firmicutes and Actinobacteria species, have been isolated from different plant compartments. Identification studies have reported that endophytic bacterial isolates may also occur as epiphytes in various plant parts (Sturz et al., 2000; Rosenblueth & Martínez-Romero, 2006; Sun et al., 2009; Santayo et al., 2016).

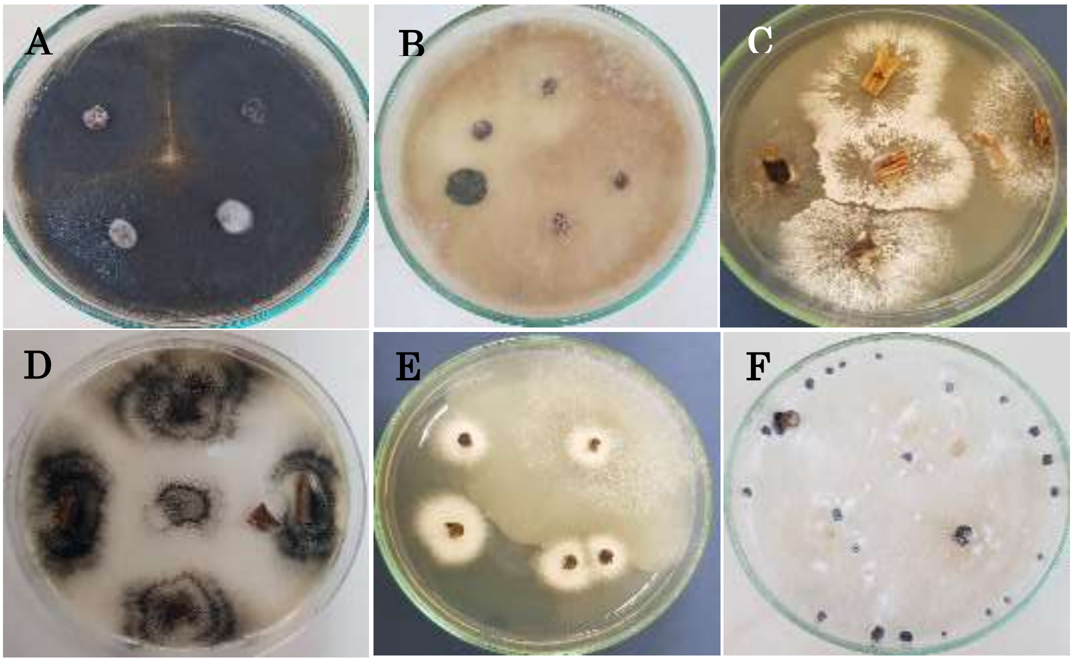

Figure 2. Mycelial growth characteristics of fungal pathogens isolated from symptomatic okra plants and seeds. (A) *Macrophomina phaseolina*, (B-C) *Rhizoctonia solani* , (D) *Verticillium dahliae*, (E) *Fusarium* spp., (F) *Sclerotinia sclerotiorum*.

Şekil 2. Simptom gösteren bamya bitkileri ve tohumlarından izole edilen fungal patojenlerinin misel gelişimleri. (A) Macrophomina phaseolina, (B-C) Rhizoctonia solani, (D) Verticillium dahliae, (E) Fusarium spp. (F) Sclerotinia sclerotiorum.

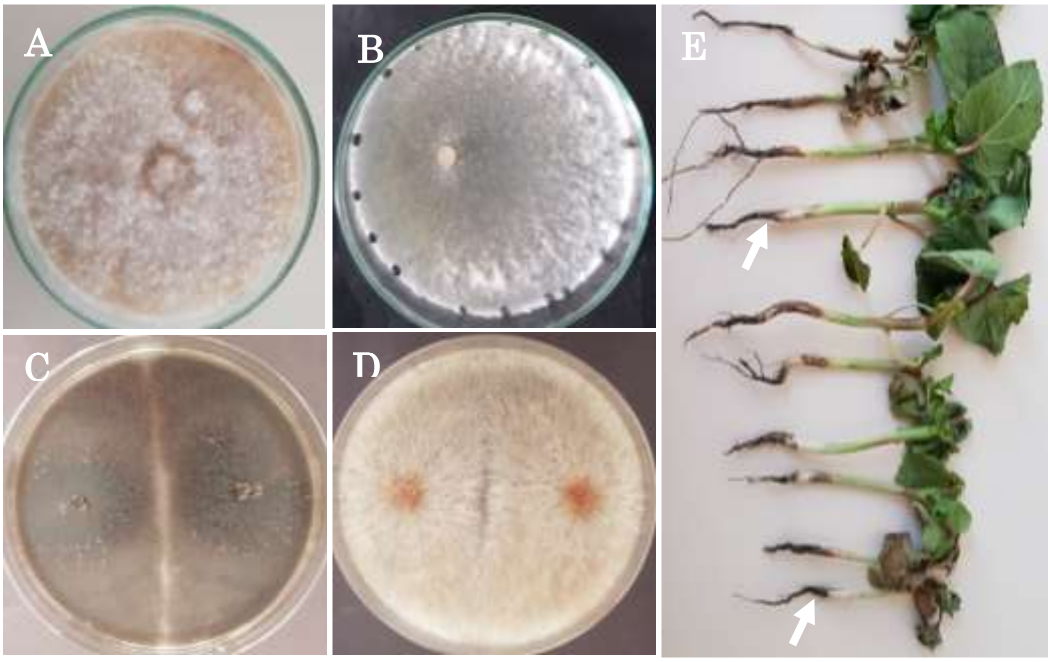

Figure 3. Morphological characteristics of fungal pathogens isolated from symptomatic okra plants and used in pathogenicity tests. (A) *Fusarium oxysporum* GAB-Fox7, (B) *Sclerotinia sclerotiorum* GAB-Ss23, (C) *Macrophomina phaseolina* GAB-Mp12, (D) *Rhizoctonia solani* GAB-Rs5 and (E) Root rot symptoms (arrows) in seedlings from pathogenicity assays.

Şekil 3. Hastalık belirtisi gösteren bamya bitkilerinden izole edilen ve patojenite testlerinde kullanılan fungal izolatlar. (A) Fusarium oxysporum GAB-Fox7*, (B) Sclerotinia sclerotiorum* GAB-Ss23*, (C) Macrophomina phaseolina* GAB-Mp12*, (D) Rhizoctonia solani* GAB-Rs5 *ve (E) Patojenite testlerinde fidelerde görülen kök çürüklüğü belirtileri (ok).*

In vitro assessment of antagonistic activity of epiphytic and endophytic bacterial isolates against mycelial growth of fungal pathogens

The antagonistic potential of 36 non-pathogenic bacterial isolates (screened via negative results in tobacco HR and potato soft rot assays) was evaluated against four major soil-borne fungal pathogens (*Fusarium oxysporum, Macrophomina phaseolina, Sclerotinia sclerotiorum, and Rhizoctonia solani*) using dual-culture assays on PDA+TSA medium (Figure 4).

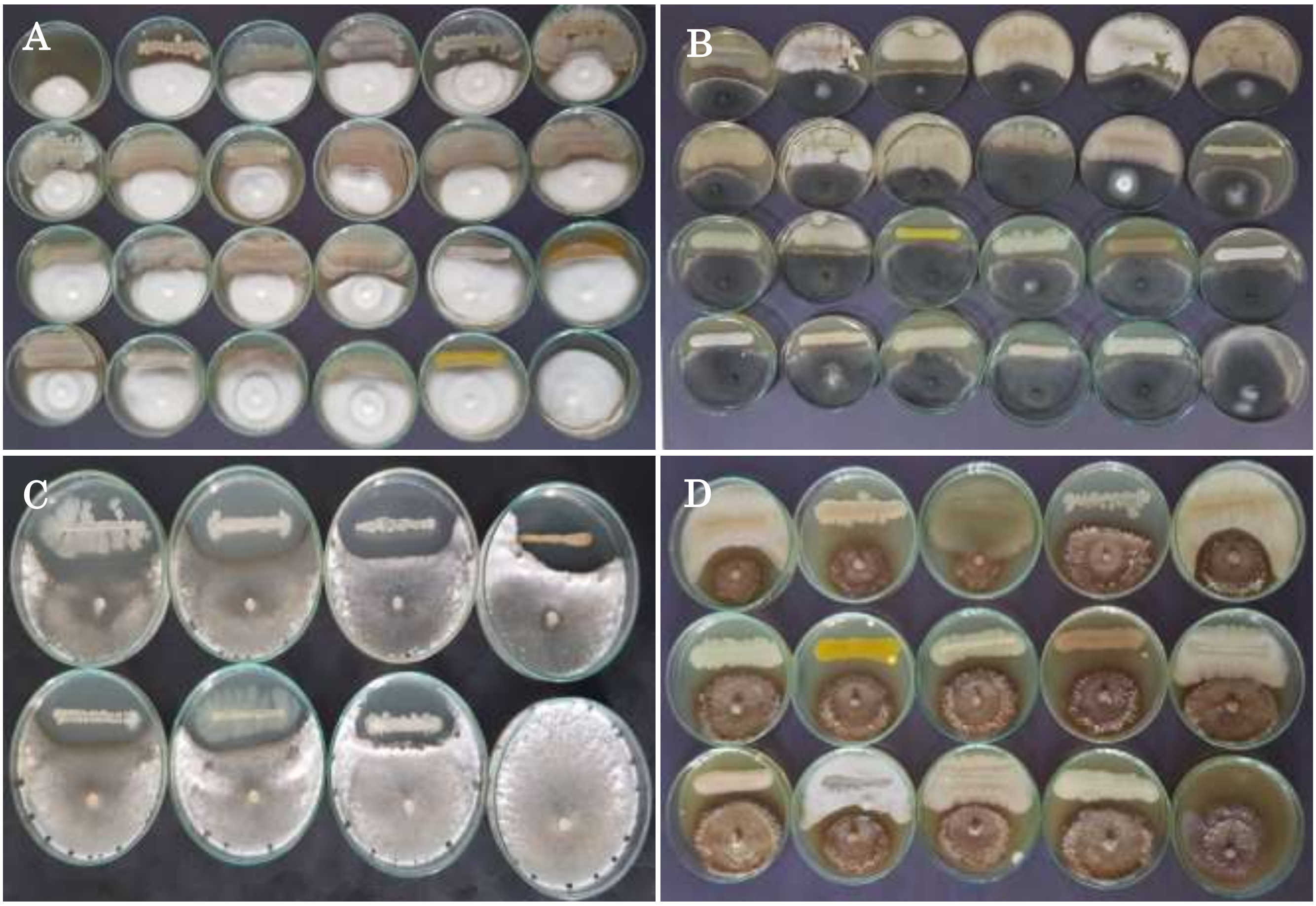

Figure 4. Dual-culture assays demonstrating mycelial growth inhibition potential of different epiphytic and endophytic bacterial isolates against (A) *Fusarium oxysporum*, (B) *Macrophomina phaseolina*, (C) *Sclerotinia sclerotiorum* and (D) *Rhizoctonia solani*

Şekil 4. Farklı epifitik ve endofitik bakteri izolatlarının (A) Fusarium oxysporum, (B) Macrophomina phaseolina, (C) Sclerotinia sclerotiorum ve (D) Rhizoctonia solani'ye karşı misel büyümesini engelleme potansiyelini gösteren ikili kültür testleri.

In the dual culture tests of 36 different endophytic and epiphytic bacterial isolates obtained from okra and closely related plants, the number of isolates that strongly inhibited (>50%) the mycelial growth of *F. oxysporum, M. phaseolina, S. sclerotiorum,* and *R. solani* was determined as follows: 33 isolates for *M. phaseolina*, 29 isolates for *R. solani*, 28 isolates for *S. sclerotiorum*, and 18 isolates for *F. oxysporum*. Additionally, the number of isolates that strongly inhibited (>50%) the mycelial growth of all four tested pathogens was found to be 17 (Table 1).

When the inhibitory effects of the 36 different bacterial isolates against the four fungal pathogens were evaluated together, the inhibition rate of mycelial growth ranged between 5.56% and 76.39%, with an average inhibition rate of 41.71% for *F. oxysporum*, the inhibition rates varied between 43.58% and 82.68%, with an average inhibition rate of 64.40% for *M. phaseolina*, the inhibition rate of mycelial growth ranged from 33.33% to 89.33%, with an average inhibition rate of 65.50% for *R. solani*, and the inhibition rates varied between 2.78% and 89.44%, with an average inhibition rate of 62.30% for *S. sclerotiorum* (Table 1).

The top three antagonistic bacterial isolates that most strongly inhibited the mycelial growth of *F. oxysporum* and fell within the same statistical group were as follows: *Bacillus cereus* B1ep, with the highest inhibition rate of 76.39%, followed by *Pseudomonas aeruginosa* B3ep, with an inhibition rate of 74.31%, and *Bacillus cereus* B11ep, with an inhibition rate of 73.61% (Table 1, Figure 4A).

Table 1. Comparative antagonistic activity of bacterial isolates against tested fungal pathogens[a].
Çizelge 1. Test edilen fungal patojenlere karşı bakteri izolatlarının karşılaştırmalı antagonistik aktivitesi[a]

	Inhibition of mycelial growth (%)			
Bacterial isolates	***Fox***	***M.p***	***S.s***	***R.s***
B. cereus B1ep	$76,39^{aB}$	$75,42^{deC}$	$89,44^{aA}$	$71,33^{g\text{-}iC}$
Pseudomonas aeruginosa B3ep	$74,31^{aC}$	$74,30^{deD}$	$82,78^{dB}$	$82,67^{bcA}$
B. cereus B11ep	$73,61^{aB}$	$73,74^{deC}$	$71,11^{fC}$	$84,00^{bA}$
B. amyloliquefaciens ssp. *plantarum* P1ep	$59,72^{dC}$	$79,33^{bcB}$	$82,22^{dA}$	$81,33^{bcA}$
B. thuringiensis B13ep	$59,72^{dC}$	$80,45^{abA}$	$84,44^{cdA}$	$68,00^{jkB}$
B. cereus B2ep	$65,28^{cBC}$	$68,72^{fC}$	$84,44^{cdA}$	$70,67^{h\text{-}jB}$
B. cereus P5en	$57,64^{deD}$	$72,07^{eC}$	$75,00^{eB}$	$80,67^{cA}$
B. amyloliquefaciens ssp. *plantarum* P3ep	$53,47^{f\text{-}hD}$	$67,60^{fC}$	$87,78^{aA}$	$74,67^{d\text{-}fB}$
B. amyloliquefaciens ssp. *amyloliquefaciens* X1en	$55,56^{efC}$	$75,42^{deAB}$	$74,44^{eB}$	$73,33^{e\text{-}hA}$
B. amyloliquefaciens ssp. *plantarum* G1en	$54,17^{fgC}$	$82,68^{aA}$	$62,78^{hiC}$	$74,00^{e\text{-}gB}$
B. subtilis B17en	$59,03^{dC}$	$72,07^{eB}$	$66,11^{gC}$	$76,00^{deA}$
B. subtilis P2en	$49,31^{iC}$	$75,98^{bB}$	$62,78^{hiC}$	$84,00^{bA}$
B. cereus B6ep	$51,39^{g\text{-}iC}$	$77,10^{cdA}$	$71,67^{fB}$	$70,00^{ijAB}$
B. cereus P7en	$65,97^{bcB}$	$72,07^{eB}$	$58,33^{j\text{-}lC}$	$73,33^{e\text{-}hA}$
B. subtilis B12ep	$63,19^{cB}$	$68,16^{fB}$	$60,56^{ijC}$	$74,00^{e\text{-}gA}$
B. subtilis subsp. *spizizenii* X4en	$48,61^{iB}$	$77,10^{cdA}$	$62,78^{hiB}$	$77,33^{dA}$
Paenibacillus polymyxa B19en	$68,75^{bA}$	$65,92^{fgB}$	$59,44^{jC}$	$62,67^{lB}$
B. thuringiensis G2en	$50,69^{hiC}$	$62,57^{hC}$	$69,44^{fB}$	$68,00^{jkA}$
B. cereus X3en	$45,14^{jC}$	$60,34^{hiB}$	$62,22^{hiB}$	$77,33^{dA}$
B. cereus B14ep	$53,47^{f\text{-}hB}$	$51,96^{k\text{-}mC}$	$86,11^{bcA}$	$45,33^{rC}$
B. cereus B5ep	$59,03^{dA}$	$60,90^{hiB}$	$58,89^{jkB}$	$53,33^{nB}$
B. horneckiae B8ep	$16,67^{mnD}$	$68,72^{fB}$	$56,67^{klC}$	$89,33^{aA}$
B. pumilus B20ep	$32,64^{kB}$	$63,69^{ghA}$	$65,56^{gA}$	$58,00^{mA}$
B. altitudinis B21ep	$27,78^{lC}$	$60,34^{hiB}$	$62,22^{hiB}$	$66,67^{kA}$
Enterobacter cloacae B10ep	$32,64^{kB}$	$55,87^{jkA}$	$56,11^{lmA}$	$54,00^{nA}$
Enterobacter cloasea P6en	$7,64^{pD}$	$62,57^{hB}$	$48,89^{pC}$	$72,67^{f\text{-}iA}$
B. cereus B16en	$6,94^{pD}$	$52,52^{lmC}$	$64,44^{ghB}$	$62,67^{lA}$
Enterobacter bugandensis B7ep	$14,58^{n\text{-}oC}$	$60,90^{hiA}$	$49,44^{opB}$	$49,33^{opA}$
Pantoea agglomerans X2ep	$5,56^{pC}$	$50,28^{mnB}$	$51,67^{noB}$	$66,00^{kA}$
Enterobacter bugandensis B18ep	$18,06^{mC}$	$51,40^{k\text{-}mB}$	$49,44^{opB}$	$52,00^{noA}$
Pseudomonas thivervalensis B9ep	$11,81^{oD}$	$51,96^{k\text{-}mB}$	$40,56^{sC}$	$65,33^{klA}$
B. altitudinis G3en	$30,56^{klC}$	$50,84^{mnB}$	$37,22^{tD}$	$46,67^{prA}$
Enterobacter bugandensis B15en	$5,56^{pC}$	$48,61^{nB}$	$53,89^{mnA}$	$40,67^{sB}$
Rhizobium radiobacter B4ep	$7,64^{pD}$	$54,19^{klA}$	$43,89^{rC}$	$38,67^{sB}$
Microbacterium maritypicum X5en	$7,64^{pB}$	$43,58^{oA}$	$47,22^{pA}$	$33,33^{tA}$
B. cereus P4ep	$31,25^{kA}$	$49,16^{mnA}$	$2,78^{uB}$	$40,67^{aA}$
Control	-	-	-	-

Fusarium oxysporum; ***M.p***: *Macrophomina phaseolina*; ***S.s***: *Sclerotinia sclerotiorum*; ***R.s***: *Rhizoctonia solani.* Green colour indicates bacterial isolates showing >70% mycelial growth inhibition; Yellow colour indicates bacterial isolates with statistically significant variations in inhibition efficacy against tested fungal pathogens. [a]Mean values followed by the same lowercase letter within each column and capital letters within each row indicate no significant difference among isolates (Tukey's HSD Test; $F_{(36,74)}$ values for *Fox, Mp, Ss and Rs* were 512.596, 225.135, 572.417,358.776, respectively; df:36, $P<0.001$

The isolate that most strongly inhibited the mycelial growth of *M. phaseolina* was *Bacillus amyloliquefaciens* ssp. *plantarum* G1en, with an inhibition rate of 82.68%. This was followed by *Bacillus thuringiensis* B13ep, with an inhibition rate of 80.45%, *Bacillus amyloliquefaciens* ssp. *plantarum* P1ep, with an inhibition rate of 79.33%, *Bacillus cereus* B6ep and *Bacillus subtilis* spizizenii X4en, both with inhibition rates of 77.1% (Table 1, Figure 4B).

The isolate with the highest antagonistic activity against *S. sclerotiorum* was *B. cereus* B1ep, showing an inhibition rate of 89.44%. This was followed by *B. amyloliquefaciens* ssp. *plantarum* P3ep, with an inhibition rate of 87.78%, *B. cereus* B14ep, with an inhibition rate of 86.11%, *B. cereus* B2ep and *B. thuringiensis* B13ep, both with inhibition rates of 84.44% (Table 1, Figure 4C).

The bacterial isolate exhibiting the strongest inhibition against *R. solani* mycelial growth was *B. horneckiae* B8ep, with an 89.33% inhibition rate. This was followed by *B. subtilis* P2en and *B. cereus* B11ep, both showing 84.00%

inhibition, *P. aeruginosa* B3ep, with an 82.67% inhibition rate, *B. amyloliquefaciens* ssp. *plantarum* P1ep, demonstrating 81.33% inhibition (Table 1, Figure 4D).

Among the tested antagonistic bacterial isolates, three strains (*B. cereus* B1ep, *P. aeruginosa* B3ep, and *B. cereus* B11ep) demonstrated high-level inhibition (≥70%) against all four pathogenic fungi (Table 1). Statistical analyses of all isolates' inhibition rates revealed significant variations in antagonistic efficacy even among isolates of the same species (Table 1). A one-way analysis of variance (ANOVA) was conducted to evaluate the inhibitory effect of different antagonistic strains on each fungal disease agent separately. The results revealed a statistically significant difference among bacterial isolates according to Tukey's HSD Test ($F_{(36,74)}$ values for *Fox, Mp, Ss and Rs* were 512.596, 225.135, 572.417, 358.776, respectively; df:36, P<0.001). The effect sizes, calculated using eta squared (η^2), were 0.996 for *Fox*, 0.991 for *Mp*, 0.996 for *Ss* and 0.994 for *Rs*. All pathogens have extremely high η^2 values (>0.99), suggesting that almost all the variability in mycelial growth inhibition is due to differences between antagonist bacterial isolates. The F-values and p-values (≤0.001) confirm that these differences are statistically highly significant. Practically, this means that the treatments applied had a profound and consistent effect on mycelial growth inhibition for each pathogen (Table 1).

Literature reviews revealed that globally, few studies have investigated biological control of soil-borne pathogens (*F. oxysporum, M. phaseolina, S. sclerotiorum, and R. solani*) affecting okra plants (Ehteshamul-Haque et al., 1990, 1996; Hasson et al., 2013; Godwin-Egein & Okereke, 2016; Okereke et al., 2016; Ashraf et al., 2018). Notably, no studies in Türkiye have explored the use of antagonistic bacteria for biological control of seed- and soil-borne pathogens in okra plants. This study, therefore, represents the first investigation of biological control strategies against pathogens affecting cultivated okra in Türkiye.

Determination of Biocontrol Mechanisms of Epiphytic and Endophytic Bacterial Isolates

The potential biocontrol mechanisms responsible for inhibiting mycelial growth (antagonism) in the isolates investigated for antagonistic activity in the trials were quantitatively or qualitatively evaluated, including their capacity to produce siderophores, protease enzymes, hydrogen cyanide (HCN), and ammonia (NH_3) (Figure 5). The obtained results are collectively presented in Table 2.

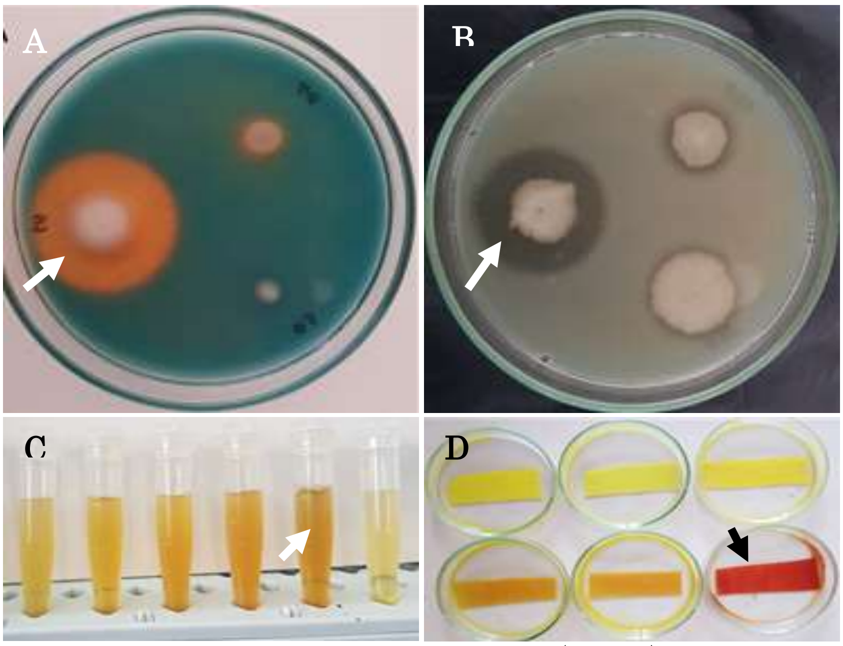

Figure 5. Production potential of different antagonistic mechanisms (arrows) by antagonistic bacterial isolates. (A) Siderophore production on CAS agar medium. (B) Protease enzymes activity on SMLBA medium. (C) Ammonia production evidenced by color transition from light yellow to dark yellow-brown in culture tubes (arrow). (D) HCN production indicated by filter paper color change from yellow to orange-red (arrow) due to increasing concentration.

Şekil 5. Antagonistik bakteri izolatları tarafından farklı antagonistik mekanizmaların (oklar) üretim potansiyeli. (A) CAS agar besiyerinde siderofor üretimi. (B) SMLBA besiyerinde proteaz enzimleri aktivitesi. (C) Kültür tüplerinde açık sarıdan koyu sarı-kahverengiye renk geçişi ile kanıtlanan amonyak üretimi (ok). (D) Konsantrasyonun artmasıyla filtre kağıdının renginin sarıdan turuncu-kırmızıya değişmesi ile gösterilen HCN üretimi (ok).

Table 2. Potential biocontrol (antagonistic) mechanisms[a] employed by different endophytic and epiphytic bacterial isolates against phytopathogens.

Çizelge 2. Farklı endofitik ve epifitik bakteri izolatlarının fitopatojenlere karşı kullandığı potansiyel biyolojik mücadele (antagonistik) mekanizmaları[a].

Bacterial isolates	Sid-İnd	Pro-ind	NH_3	HCN	%MGI
Pseudomonas aeruginosa B3ep	2,11^{hj}	1,61^{f}	+++	+++	78,52
Bacillus cereus B1ep	2,07^{hi}	1,03^{b}	+++	-	78,15
B. amyloliquefaciens ssp plantarum P1ep	1,19^{bd}	1,15^{c}	+++	-	75,65
B. cereus B11ep	1,64^{fg}	1,15c	+++	-	75,62
B. thuringiensis B13ep	1,93^{h}	1,73^{g}	+++	-	73,15
B. amyloliquefaciens ssp plantarum P3ep	1,31^{de}	1,14^{c}	++	-	70,88
B. amyloliquefaciens ssp, amyloliquefaciens X1en	1,22^{bd}	0,00^{a}	+++	-	69,69
B. amyloliquefaciens ssp, *plantarum* G1en	1,08^{bc}	1,08^{bc}	++	-	68,41
B. subtilis P2en	1,15^{bd}	1,46^{de}	+++	-	68,02
B. cereus P7en	1,44^{ef}	1,39^{d}	+++	-	67,43
B. subtilis B12ep	1,03^{b}	1,03^{b}	++	-	66,48
B. subtilis subsp, *spizizenii* X4en	0,00^{a}	1,52^{e}	++	-	66,46
Paenibacillus polymyxa B19en	1,18^{bd}	0,00^{a}	±	-	64,20
B. cereus B14ep	1,68^{g}	1,73^{g}	+++	-	59,22
B. horneckiae B8ep	1,28^{ce}	0,00^{a}	++	±	57,85
B. pumilus B20ep	2,28^{jl}	2,24^{h}	++	++	54,97
Enterobacter cloacae B10ep	2,74^{m}	0,00^{a}	+	-	49,66
B. cereus B16en	1,60^{fg}	1,05^{b}	++	-	46,64
E. bugandensis B7ep	2,13^{ik}	0,00^{a}	+++	+	43,56
Pantoea agglomerans X2ep	1,69^{g}	0,00^{a}	+	-	43,38
Pseudomonas thivervalensis B9ep	2,30^{jk}	0,00^{a}	+	-	42,42
B. altitudinis G3en	1,24^{cd}	0,00^{a}	++	-	41,32
B. altitudinis B21ep	1,12^{bd}	1,03^{b}	+++	-	41,32
Rhizobium radiobacter B4ep	1,13^{bd}	0,00^{a}	++	-	36,10
Microbacterium maritypicum X5en	1,11^{bd}	2,61^{i}	±	-	32,94

Sid-Ind: Siderophore production index; **Pro-Ind:** Protease activity index; **NH_3:** Ammonia production; **HCN:** Hydrogen cyanide production; **%MGI:** Percentage mycelial growth inhibition; **–** : No color change (negative result); **±** : Trace production (faint color change); **+** : Low production (weak positive); **++** : Moderate production; **+++** : High production (intense color change)
[a]Within a given column, mean values followed by the same lowercase letter are not statistically significant (Tukey's HSD test, $P < 0.01$).

As shown in Table 2, examination of the siderophore index values formed on CAS agar medium for 25 bacterial isolates with high inhibition rates against the four pathogens revealed that the highest siderophore production activity was demonstrated by *Enterobacter cloacae* B10ep with an index value of 2.74, followed by *P. thivervalensis* B9ep (2.30) and *B. pumilus* B20ep (2.28) (Table 2). Among the bacterial isolates showing the highest inhibition rates against the pathogens, *P. aeruginosa* B3ep (2.11), *B. cereus* B1ep (2.07), and *B. subtilis* B12ep (1.03) also exhibited high siderophore production activity (Table 2).

Secretion of lytic enzymes, such as cellulase, protease, and chitinase enable endophytic bacteria to penetrate plant tissues and establish stable colonies, providing a competitive advantage to bacteria possessing this capability (Compant et al., 2005). Among the 25 tested isolates, 17 isolates produced protease enzymes at varying levels. The highest protease activity was observed in *Microbacterium maritypicum* X5en with an index value of 2.61, followed by *Bacillus pumilus* B20ep (2.24), *Bacillus thuringiensis* B13ep (1.73), and *Bacillus cereus* B14ep (1.73).

The potential of bacterial isolates to produce volatile compounds - ammonia (NH_3) and hydrogen cyanide (HCN), as another antagonistic mechanism, was qualitatively assessed. As shown in Table 2, bacterial isolates demonstrating high antagonistic activity (72.22-82.78%), including *Bacillus cereus* P7en, *Bacillus altitudinis* B21ep, *Enterobacter bugandensis* B7ep, *Bacillus cereus* B14ep, *Pseudomonas ae*ruginosa B3ep, *Bacillus pumilus* B20ep, *Bacillus subtilis* P2en, and *Bacillus horneckiae* B8ep, were found to produce varying concentrations of ammonia and HCN in the culture medium.

Certain beneficial bacteria found in plants produce hydrogen cyanide (HCN), a toxic gas with antimicrobial effects against pathogens. HCN inhibits pathogen development by blocking cellular respiration and energy production in pathogen cells (Schippers et al., 1990). Among the tested antagonistic bacterial isolates showing >70% average effectiveness in inhibiting mycelial growth of four fungal pathogens, Pseudomonas aeruginosa B3ep was found to produce siderophores, protease, ammonia, and HCN. In contrast, isolates *Bacillus cereus* B1ep, *Bacillus amyloliquefaciens* ssp. *plantarum* P1ep, *Bacillus cereus* B11ep, *Bacillus thuringiensis* B13ep, and *Bacillus amyloliquefaciens* ssp. *plantarum* P3ep tested positive for other mechanisms excluding HCN production (Table 2).

Determination of plant growth-promoting mechanisms of epiphytic and endophytic bacterial isolates

The potential plant growth-promoting mechanisms of bacterial isolates showing antagonistic activity in the trials, including indole acetic acid (IAA) production and phosphate solubilization capacity, were quantitatively determined (Table 3, Figure 6). As shown in Table 3, all 29 tested isolates produced IAA at concentrations ranging between 3.18 and 85.59 µg/ml. Among the tested isolates, *Enterobacter bugandensis* B7ep showed the highest IAA production (85.59 µg/ml), followed by *E. cloacae* B10ep (81.09 µg/ml) and *R. radiobacter* B4ep (73.15 µg/ml). The lowest IAA production (3.18 µg/ml) was observed in *Paenibacillus polymyxa* B19en. Although demonstrating high antagonistic effects, isolates *B. cereus* B1ep, *B. subtilis* B12ep and *P. aeruginosa* B3ep were found to produce relatively low levels of IAA (Table 3).

Table 3. Potential mechanisms[a] employed by different epiphytic and endophytic antagonistic bacterial isolates in promoting plant growth.

Çizelge 3. Bitki büyümesini teşvik etmek için farklı epifitik ve endofitik antagonistik bakteri izolatları tarafından kullanılan potansiyel mekanizmalar[a].

Bacterial isolates	IAA (µg/ml)	PSI
Enterobacter bugandensis B7ep	**85.59**^{z}	1.35$^{c\text{-}f}$
E. cloacae B10ep	**81.09**^{y}	1.25$^{b\text{-}e}$
Rhizobium radiobacter B4ep	**73.15**^{w}	0.00^{a}
Bacillus subtilis subsp. *spizizenii* X4en	65.48^{v}	0.00^{a}
Pantoea agglomerans X2ep	51.76^{u}	1.94^{h}
Microbacterium maritypicum X5en	27.66^{u}	0.00^{a}
B. cereus B2ep	24.44^{t}	1.49$^{e\text{-}g}$
B. altitudinis B21ep	24.20^{s}	1.13$^{b\text{-}d}$
Pseudomonas thivervalensis B9ep	23.82^{r}	**2.50**^{j}
B. cereus P7en	15.31^{p}	**2.60**^{j}
B. horneckiae B8ep	14.25^{o}	0.00^{a}
B. pumilus B20ep	14.19^{o}	0.00^{a}
B. cereus B16en	12.56^{n}	1.40^{df}
B. cereus B14ep	10.24^{m}	1.71^{fg}
B. amyloliquefaciens ssp. *plantarum* P3ep	7.96^{l}	1.05^{b}
B. thuringiensis G2en	7.52^{k}	0.00^{a}
B. subtilis P2en	7.26^{j}	0.00^{a}
B. amyloliquefaciens ssp. *amyloliquefaciens* X1en	5.72^{g}	0.00^{a}
B. amyloliquefaciens ssp. *plantarum* G1en	6.98^{i}	0.00^{a}
B. altitudinis G3en	4.15^{d}	1.60^{ef}
Pseudomonas aeruginosa B3ep	3.65^{c}	**2.22**^{i}
Paenibacillus polymyxa B19en	3.18^{b}	1.17$^{b\text{-}d}$

IAA: indole acetic acid; **PSI**: phosphate solubilisation index

[a]Mean values within columns followed by the same letter are not statistically significant (Tukey's HSD Test, P < 0.01).

The phosphate solubilization potential of the bacterial strains used in the study was determined using Pikovskaya's agar (PVK) medium. The values were calculated as the ratio of the phosphate solubilization zone diameter to the bacterial colony diameter (Table 3). Among the 24 tested bacterial isolates, 11 showed no phosphate solubilization activity, while 13 isolates exhibited solubilization indices ranging from 1.13 to 2.60 (Table 3). The most effective phosphate-solubilizing isolates were *Bacillus cereus* P7en with a solubilization index of 2.60, *Pseudomonas thivervalensis* B9ep (2.50), and *Pseudomonas aeruginosa* B3ep (2.22).

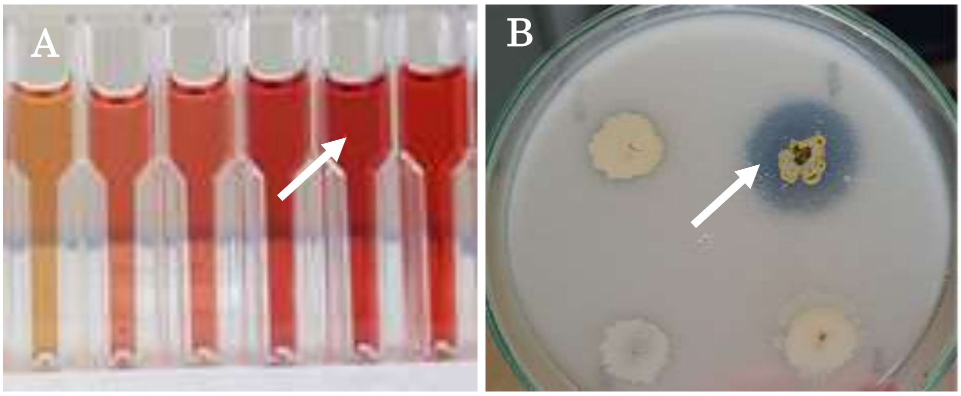

Figure 6. Determination of IAA production and phosphate solubilization potential of different antagonistic bacterial isolates on PVK medium (arrows). (A) IAA production assay. IAA production was assessed in LB medium. Colorimetric changes from yellow to red pink indicate varying concentrations of IAA production. (B) Phosphate solubilization activity on PVK medium. Arrow indicates the characteristic solubilization zone.

Şekil 6. Farklı antagonistik bakteri izolatlarının IAA üretimi ve PVK besiyerinde fosfat çözünürleştirme potansiyelinin belirlenmesi (ok). (A) IAA üretim testi. IAA üretimi LB besiyerinde değerlendirildi. Sarıdan kırmızı-pembeye renk değişimi, IAA üretiminin farklı konsantrasyonlarını gösterir. (B) PVK besiyerinde fosfat çözünürleştirme aktivitesi. Ok, karakteristik çözünürleştirme bölgesini gösterir.

In biological control studies, the most extensively researched antagonistic bacterial isolates predominantly belong to species such as *Bacillus subtilis, B. amyloliquefaciens, B. pumilus, B. licheniformis, B. cereus,* and *B. thuringiensis*, which are known to produce various antimicrobial compounds (Stein, 2005). Previous biocontrol research has demonstrated that these *Bacillus* species inhibit disease initiation and development through the production of antimicrobial metabolites including iturin, surfactin, lichenysin, and fengycin (Tsuge et al., 1999; Kim et al., 2004; Huszcza & Burczyk, 2006; Yang et al., 2020). Several key factors, such as pathogens cannot develop resistance against bacterial toxins, they have capacity to produce diverse antimicrobial metabolites, form environmentally resistant endospores under unfavorable conditions, contribute to the prominence of *B. subtilis* as the most studied and preferred species in biocontrol formulations. Beyond *Bacillus* species, representatives from *Agrobacterium* (syn. *Rhizobium*) and *Pseudomonas* genera also constitute important taxa investigated in biocontrol research (Fravel, 2005). Previous studies on BCA and PGP endophytic and epiphytic bacterial isolates, belonging to different *Bacillus, Pseudomonas,* Paenobacillus, *Enterobacter* spp, suppressed soil-borne fungal diseases producing significant amounts of antagonistic and PGP mechanisms such as siderophore, protease, HCN, NH_3, IAA and solubilization of macro/micro nutrient elements have reported that (Aktan & Soylu, 2020; Kara et al., 2020; Soylu et al., 2021; Kara & Soylu, 2022; Kara et al., 2023).

Significant variations were observed in the pathogen inhibition rates among bacterial isolates belonging to the same species. As previously reported in numerous studies (Araujo et al., 2005), these differences may be attributed to the genetic composition of the host plant where the antagonistic bacteria developed, environmental conditions of the host's growth habitat and genetic characteristics of the bacterial isolates themselves (Soylu et al., 2021).

CONCLUSION

Antagonistic bacteria used in biological control offer several advantages over chemical methods, including absence of phytotoxic effects, no induction of pathogen resistance, effective pathogen suppression without environmental harm. Key bacterial genera employed in biocontrol such as Bacillus spp., Pseudomonas spp., and Enterobacter spp. demonstrate dual functionality by pathogen suppression through production of antagonistic and plant growth-promoting traits. This study represents the first comprehensive investigation in Türkiye evaluating the biocontrol efficacy of antagonistic BCA bacteria, their mechanisms of action and their potential against seed- and soil-borne fungal pathogens in okra plants. BCA isolates from Bacillus spp., Pseudomonas spp., and Enterobacter spp. showing strong antagonistic activity (siderophore, protease, and ammonia production) and significant plant growth-promoting traits (IAA and phosphate solubilization) have high potential as chemical alternatives for disease management. The study highlights these bacterial isolates as promising biocontrol agents for sustainable okra cultivation.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

Ethics approval and consent to participate

This article lacks any study related to human or animal participants performed by any of the authors.

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