Yuzuncu Yil University Journal of Agricultural Sciences, Volume: 35, Issue: 2, 30.06.2025

Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Antifungal Activity of Endophytic *Bacillus* spp. Bacteria and Its Effect on Root and Coleoptile Length during Germination Period

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Article Info

Received: 13.12.2024
Accepted: 17.04.2025
Online published: 15.06.2025
DOI: 10.29133/yyutbd.1601276

Keywords

Antagonist,
Biological control,
N. dimidiatum

Abstract: In this study, endophytic bacteria were isolated from roots and crowns of rosemary (*Rosmarinus officinalis* L.), olive (*Olea europaea* L.), and loquat (*Eriobotrya japonica* L.) plant samples. The morphological and physiological properties of nine isolated endophytic bacteria were determined. All isolates were identified as Gram-positive, oxidase-positive and catalase-positive. Amylase, cellulase, and carbohydrate tests gave positive results. Antagonistic activities of the isolates against fungal pathogens varied between 85.7% and 52.9% against *Fusarium culmorum* and between 86.0% and 65.1% against *Neoscytalidium dimidiatum*. BMBA2 isolate gave the best results both in Petri dish antagonistic activity and in wheat seed germination in terms of root length and coleoptile length. BMBA2 isolates gave the best results with a coleoptile length of 7.58 cm and root length of 8.33 cm. In wheat seeds treated with *F. culmorum* and bacteria, the BMBA2 isolate gave the best result with a coleoptile length of 6.98 and a root length of 7.30 cm. For the identification of bacteria, in vitro BiBA1 and ND3BA were determined as *Bacillus amyloliquefaciens* subsp. *plantarum*; BiBA2 and YDBA as *Bacillus subtilis*; NDBA, ND2BA, BMBA1, BMBA2, and BMBA3 as *Bacillus mojavensis*. Since this is the first study to use endophytic *Bacillus mojavensis* as a biological agent against *F. culmorum* and *N. dimidiatum* pathogens, the results obtained from this study are thought to be important and promising in terms of application.

To Cite: Güler Güney, İ, 2025. Antifungal Activity of Endophytic *Bacillus* spp. Bacteria and Its Effect on Root and Coleoptile Length during Germination Period. *Yuzuncu Yil University Journal of Agricultural Sciences*, 35(2): 334-349. DOI: https://doi.org/10.29133/yyutbd.1601276

1. Introduction

Wheat production increased by 11.4% compared to the previous year and reached 22 million tons of wheat. Wheat production accounts for 28.3% of the production of cereals and other plant products (TURKSTAT, 2023). Wheat is an annual plant and a staple food widely used in many countries of the world. Unfortunately, wheat grown under field conditions is exposed to various factors such as biotic and abiotic factors such as high salt concentrations, water scarcity, and high temperatures that may affect it at different developmental stages (Ghazala et al., 2023).

In recent times, agriculture has been exposed to biotic factors originating from pathogens, stress, and abiotic factors such as salinity, drought, and extreme temperatures. These problems have been exacerbated by the continuous increase in population and the combined effects of extreme climate events such as drought, floods, and high temperatures have also led to significant decreases in crop yields. It has also faced problems such as excessive use of chemical pesticides and fertilizers and the reduction of biodiversity in agriculture. In addition, the excessive use of chemical compounds has caused pathogen resistance and environmental pollution (Bódalo et al., 2023). The use of synthetic pesticides has increased since the 1940s, and pesticides damage non-target plants and the environment and can cause adverse health effects (Hernández et al., 2013; Bernardes et al., 2015; Carvalho, 2017; Tudi et al., 2021). Therefore, it is necessary to develop alternative methods to reduce the resistance of agricultural products to chemical compounds.

The *Neoscytalidium dimidiatum* pathogen has been widely distributed in different plants and has spread to 37 countries. The presence of this pathogen has been detected in various regions, including where *N. dimidiatum* pathogen was observed, 30 in Türkiye (Akgül et al., 2019; Güney et al., 2021; Alkan et al., 2022; Özer et al., 2022; Derviş and Özer, 2023), 47 in Iran (Ahmadpour et al., 2023; Esmaeil et al., 2023), 14 in Brazil (Monteles et al., 2020; Mello et al., 2021) 16 in South America (Arrieta-Guerra et al., 2021; Espinoza-Lozano et al., 2023), 18 in Malaysia and 6 in China (Ismail et al., 2021; Sha et al., 2022). The United States, particularly in California, displays a substantial presence, with 38 occurrences (French, 1989; Farr et al., 2005). Middle Eastern countries have also documented the detection of this pathogen in 6 different plants in Iraq (Al-Tememe et al., 2019; Abdulrahman and Haleem, 2023) and 9 in Oman (Al-Sadi et al., 2014). The distribution indicator of this pathogen shows that *N. dimidiatum* poses a global threat. This pathogen, seen in different examples in Türkiye, emphasizes that it is an important pathogen that needs to be addressed in the region (Derviş and Özer, 2023). Symptomatic expression is particularly pronounced in perennial plants such as dragon fruits (Chuang et al., 2012; Espinoza-Lozano et al., 2023; Khoo et al., 2023; Salunkhe et al., 2023), citrus fruits (Polizzi et al., 2009; Mayorquin et al., 2016), grapevines (Al-Saadoon et al., 2012; Rolshausen et al., 2013; Oksal et al., 2019), pines (Türkölmez et al., 2019a), stone fruits (Nouri et al., 2018; Oksal et al., 2020), *Ficus* species (Güney et al., 2022), pistachios (Derviş et al., 2019), and willows (Hashemi and Mohammadi, 2016), leading to reduced yields and shorter life spans (Türkölmez et al., 2019b). It has also been stated that *N. dimidiatum* has the potential to spread from seeds, soil, and air and to persist in the soil (Çiftçi et al., 2023; Güney et al., 2023).

Investigating the potential of environmentally friendly biological control agents and environmentally friendly biopesticides in controlling *N. dimidiatum* and related pathogens may provide sustainable alternatives for disease management. The use of biological control agents with broad-spectrum bioactive metabolites is recommended as an alternative to chemical pesticides against fungal phytopathogens. It has been reported that various bacterial genera such as *Bacillus* sp., *Pseudomonas* sp., and *Streptomyces* sp. are widely used in biocontrol studies (Albayrak, 2019; Singh et. al., 2024). It has been observed that the *F. culmorum* pathogen causes serious decreases in both yield and quality in wheat production regions, thereby further worsening the difficulties faced by the wheat production sector (Gökçe and Kotan, 2016; Bozoğlu et al., 2022). Güler Güney et al. (2024) reported that the endophytic *Stenotrophomonas rhizophila*, *Pseudomonas putida,* and *Pseudomonas orientalis* (EY1+EM9+MM21) combination gave the best results against *F. culmorum* in terms of disease severity, plant height, fresh weight, dry weight, root fresh weight and root dry weight in wheat plants. They reported that various endophytic bacteria can be used against *F. culmorum*.

Bacteria have different mechanisms to manage plant biocontrol, such as antagonistic activities, production of secondary metabolites or some lytic enzymes, competition, and induction of plant resistance. It is known that most endophytes are effective in plant growth and that without them, plants have difficulty coping with biotic and abiotic stresses (Santoyo et al., 2016). Endophytes play critical roles in plant ecology, evolution, and development, including nitrogen fixation (Carvalho et al., 2014), growth promotion, and production of antimicrobial substances (Singh et al., 2017), and are also considered important in increasing plant resistance to plant stress (Li et al., 2019) and remediating environmental pollution (Barik et al., 2021). Endophytes (bacteria and fungi) constitute a group of endosymbiotic microorganisms that are ubiquitous in nature. It is known that they live in the plant endosphere and do not show any significant harmful effects on any host plant (Compant et al., 2021; Hazarika et al., 2021). Studies have stated that endophytes facilitate nutrient uptake, promote growth, are effective against pathogenic microorganisms, and activate the defense systems of plants by increasing the production of secondary metabolites (Aeron et al., 2020; Patel et. Al. 2024).

Bacillus is a Gram-positive, endospore-forming, rod-shaped, aerobic, or facultative anaerobic bacterium (Logan and De Vos, 2009). *Bacillus* species are among the most widespread bacteria in nature and constitute one of the most important microbial sources in studies of plant-endophyte interactions (Izumi, 2011). Endophytic *Bacillus* species are generally preferred over other bacteria in biological control studies due to their ability to produce a significant number of secondary metabolites and hydrolytic enzymes and their biological and environmentally friendly properties (Sari et al., 2006).

Bacillus sp. appears to be more effective as a biological control agent due to its resistance to adverse environmental conditions, and its antagonistic effect against a wide range of pathogens (Shafi et al., 2017). According to the latest studies, *B. mojavensis, B. subtilis, B. amyloliquefaciens, B. thuringiensis,* and *B. polymyxa* species appear to be effective as biological agents (Bacon and Hill, 1996; Bacon and Hinton, 2002; Snook et al., 2009; Wang et al., 2019; Roy et al., 2021). *Bacillus mojavensis* is known to produce certain biosurfactants such as surfactin A, phenytoin, and iturin derivatives, as well as hydrolytic enzymes that play a role in antifungal activities. *Bacillus mojavensis* is preferred as a biological control agent due to its ability to inhibit many pathogens (Xiao et al., 2009; Jasim et al., 2016).

In this study, the antifungal activities of Endophytic *Bacillus* sp. isolates against *F. culmorum* and *N. dimidiatum* pathogens and the effects on some growth parameters of wheat seeds inoculated with *F. culmorum* and endophytic bacteria were investigated and effective results were obtained.

2. Material and Methods

2.1. Isolation of endophytic bacteria

Rosemary, olive, and loquat plant samples (1-2 cm long) were disinfected from the root and root collar parts and left to grow in Nutrient agar (NA)-containing media (Zvyagintsev, 1991). All representative isolates, bacteria taken from 24-48 hours fresh cultures for other studies, were cultured on NA-containing slant agar media. For long-term storage, all representative isolates were re-purified into NA medium and stored at –80 °C in 30% glycerol for *in vitro* tests.

2.2. Procurement and storage of pathogenic isolates

Fusarium culmorum and *N. dimidiatum* pathogens were obtained from the Mardin Artuklu University laboratory. Fungal pathogens were grown in Petri dishes on Potato dextrose agar (PDA) medium at 24 °C and stored in a tilted agar medium containing PDA at +4 ºC until used.

2.3. Disinfection of seeds

The seeds used in the experiment were surface disinfected before the experiment. The seeds were kept in sdH_2O for 5 minutes, 75% ethanol for 30 seconds, and 0.5% NaOCl for 1 minute to perform surface disinfection. Then, they were washed twice in sdH_2O and dried in a laminar cabinet. They were placed in sterile containers (Gargouri-Kammoun et al., 2009).

2.4. Examination of morphological characteristics of bacteria

Gram staining: Gram staining of isolates was performed (Demirbağ and Demir, 2005). Catalase: Isolates were grown in nutrient broth medium at 28 °C for 2 days. Foaming status will be checked by adding 3% H_2O_2 to the isolates. If there is foaming, it is evaluated as positive (+), if not, it is evaluated as negative (-) (Holt et al., 1994). Oxidase: A loopful of freshly developed samples from bacterial isolates were taken and spread on blotting paper, then oxidase was poured and color change was observed. Blue color formation was concluded as positive (Holt et al., 1994).

2.5. Carbohydrate tests

The growth of the isolates was evaluated at 28 °C for 3, 7, and 14 days after being separately filtered through a 0.45 μm filter into sterile mineral salt broth according to the method of Ji and Wilson (2002).

2.6. Enzymatic activity

In all enzyme experiments, cultures of the isolates prepared according to Mc Farland No. 5 were used. All enzyme experiments were performed with 3 replicates. Amylase (Hydrolysis of Starch): Isolates were streaked onto Petri dishes containing starch agar and incubated at 28 °C for 2 days. Lugol solution was dropped on the colonies that developed on the medium and the formation of a bright color was evaluated as positive for starch hydrolysis, and the formation of a blue-black color was evaluated as negative (Egamberdieva et al., 2008). Cellulase: 1 ml was taken from the sterilized B and D (Solution B: $MgSO_4.7H_2O$ 1M; Solution D: $CaCl_2$ 7.5% (V/W) solutions and added to the sterilized A+C (Solution A: NaCl 0.25 g, carboxymethylcellulose 2.5 g, K_2HPO_4 1.5 g, distilled water 400 ml; Solution C: Na_2HPO_4 3 g, yeast extract 0.5 g, glycerol 2.5 g, NH_4Cl 0.5 g, agar 6.5 g, distilled water 100 ml) solutions. Isolates were subjected to streaking onto this composite medium and were subsequently incubated at 28 °C for a duration of 96 hours. At the end of incubation, 0.1% Congo red solution was dropped on the colony and kept for 20 min. Petri dishes were washed with 1 N NaCl solution. Those showing a clear zone around the colony were evaluated as cellulase activity positive (Egamberdieva et al., 2005).

2.7. Antagonistic activity

The effects of endophytic bacterial isolates with detected antagonistic effects against *F. culmorum* and *N. dimidiatum* pathogens were performed in vitro in Petri dishes. The antagonistic activities of bacteria against pathogens were determined. The antagonistic activity of bacteria in Petri dishes against pathogens was calculated according to the formula below (Ahmad et al., 2008).

$$\%RI = R - r / R \times 100 \tag{1}$$

R: : Development of pathogenic fungus on the bacteria-free side
r : Development of pathogenic fungus towards bacteria
%RI : Inhibition rate

2.8. Effect of isolates on wheat seed germination period characteristics and examination of their effect against *Fusarium culmorum* pathogen

Two different applications were examined for their effect on seed germination and growth as well as their effect against the *F. culmorum* pathogen. In the first application, 144 wheat seeds were used in an endophytic bacterial suspension (10^7 CFU ml^{-1}) with 4 wheat varieties in each Petri dish and 36 Petri dishes (9 × 4) for each isolate and four replicates. For the control, 160 wheat seeds and 40 Petri dishes were used with 16 seeds (4 × 4) by applying distilled water to the seeds. Wheat seeds were sterilized and dried in a hood; dried sterilized seeds were placed on a sterile agar plate for 24 h at 21 °C to allow germination. Embryos were then inoculated with endophytic bacteria suspension (Simons et al., 1996).

In the second application, in order to examine its effect on the *F. culmorum* pathogen, only *F. culmorum* was planted in Petri dishes with 4 replicates as control, and wheat seeds in other Petri dishes were inoculated with nine bacteria, and *F. culmorum*, and in order to investigate their effectiveness against the disease, 144 wheat seeds (9*4*4) were contaminated with *F. culmorum* in 40 Petri dishes, and 16 wheat seeds (4*4) were left as control. Wheat seeds were sterilized and dried in a hood; dried sterilized seeds were placed on a sterile agar plate for 24 h at 21 °C to allow germination. Embryos were then inoculated with *F. culmorum* by adding 10 ml of a conidial suspension (3×10^5 spores ml^{-1} H_2O) to each embryo (Jaber, 2018).

2.9. Statistical analysis

Antagonistic activities of endophytic bacteria and statistical analyses of data obtained from seed germination experiments in Petri dishes were evaluated according to the JMP program Tukey's Test Method. The means and applications were considered significant when $P<0.01$. Differences analyses of groups were subjected to one-way ANOVA t-test analysis of variance according to Tukey's HSD test.

3. Results and Discussion

3.1. Examination of morphological characteristics and phenotypic tests of bacteria

Nine of the most effective isolates were selected in the endophytic bacteria isolation process. All endophytic bacteria gave positive results for oxidase and catalase. All isolates showed growth in carbohydrate tests. In enzyme tests, all were evaluated positive in amylase and cellulase tests (Table 1, Figure 1).

Table 1. Phenotypic tests of endophytic bacteria

Isolates	Oxidase	Catalase	Amylase	Cellulase	Carbohydrates (glucose, fructose, mannitol, maltose, m-inositol
BiBA1	+	+	+	+	+
BiBA2	+	+	+	+	+
NDBA	+	+	+	+	+
ND2BA	+	+	+	+	+
ND3BA	+	+	+	+	+
YDBA	+	+	+	+	+
BMBA1	+	+	+	+	+
BMBA2	+	+	+	+	+
BMBA3	+	+	Z	+	+

+: Positive; Z: Weak.

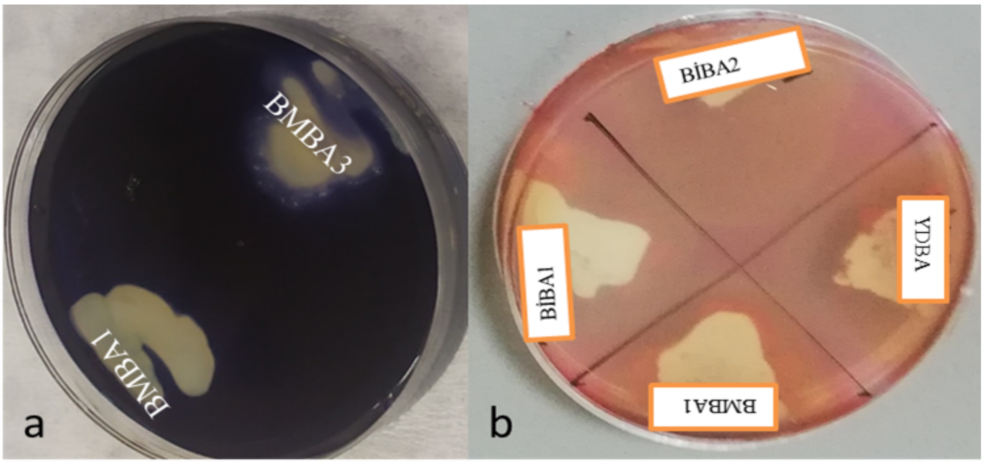

Figure 1. a) Amylase activity of endophytic bacteria b) Cellulase activity of endophytic bacteria.

The 9 isolates tested gave promising results. Camele et al. (2019) also found that it is an endophytic bacterium with fungicidal effects against some phytopathogens in their study with *B. mojavensis*. They stated that bioactive secondary metabolites produced by *B. mojavensis* may have promising applications in the agricultural, food industry, and clinical fields (Camele et al., 2019).

3.2. Antagonistic activity

The isolates' effectiveness against *F. culmorum* varied between 85.7 and 52.9%. Inhibition rates against *N. dimidiatum* varied between 85.7 and 65.1%. BMBA2 isolate was selected as the most effective isolate against *F. culmorum* with 85.7%. YDBA isolate was selected as the most effective isolate against *N. dimidiatum* with 86.0% of BMBA2 isolate (Table 2).

Table 2. Antagonistic activities of endophytic bacteria against *Fusarium culmorum* and *Neoscytalidium dimidiatum*

Isolates	**%RI *F.c***	**%RI *N.d***
BiBA1	52.90^{h}	65.10^{g}
BiBA2	57.10^{g}	74.40^{f}
NDBA	64.30^{e}	81.40^{c}
ND2BA	62.10^{f}	74.40^{f}
ND3BA	71.40^{c}	76.70^{e}
YDBA	78.60^{b}	86.00^{a}
BMBA1	65.70^{d}	79.10^{d}
BMBA2	85.70^{a}	83.70^{b}
BMBA3	64.30^{e}	79.10^{d}
Mean	**66.9**	**77.8**
SE	**0.05**	**0.12**

F.c: Fusarium culmorum; N.d: Neoscytalidium dimidiatum; %RI: Inhibition rate SE: Std Error.
There is no statistically significant difference between values.

Endophytic bacteria *F. culmorum* were found to be effective in their antagonistic activities against *N. dimidiatum* (Figure 2, Figure 3). According to the Tukey multiple comparison test, the differences between the means of the effects of the bacteria were found to be significant (Table 2).

Table 3. Variance analysis of the antagonistic activities of endophytic bacteria

Fusarium ***culmorum***					
Sources of Variation	**DF**	**SS**	**MS**	**F Value**	***P***
Isolates	8	3362.48	420.31	43647.58	<0.0001***
Rep.	3	0.0089	0.003	0.3077	0.8196
Error	24	0.2311	0.010		
General	35	3362.72			
Neoscytalidium ***dimidiatum***					
Sources of Variation	**DF**	**SS**	**MS**	**F Value**	***P***
Isolates	8	1216	152	2533.3	<.0001***
Rep.	3	0.72	0.24	4	0.0192
Error	24	1.44	0.06		
General	35	1218.16			

DF: Degrees of freedom SS: Sum of squares MS: Mean of squares.
*** Difference between bacteria according to Tukey LSD Significant according to $\alpha = 0.05$.

According to the one-way analysis of variance (ANOVA) obtained from the observation values, the differences between the antagonistic activities of the bacteria were found to be statistically significant (Table 3).

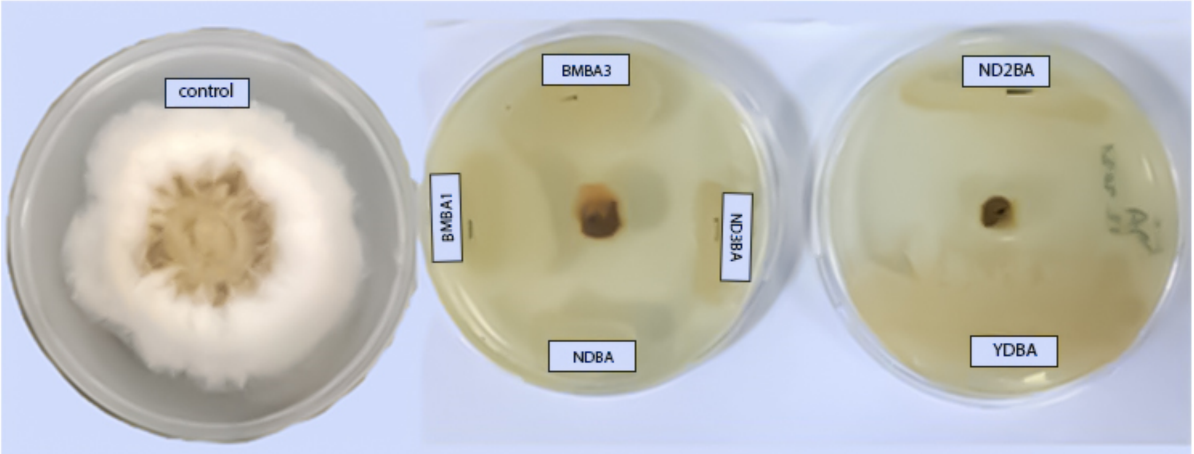

Figure 2. Antagonistic activities of endophytic bacteria against *Fusarium culmorum.*

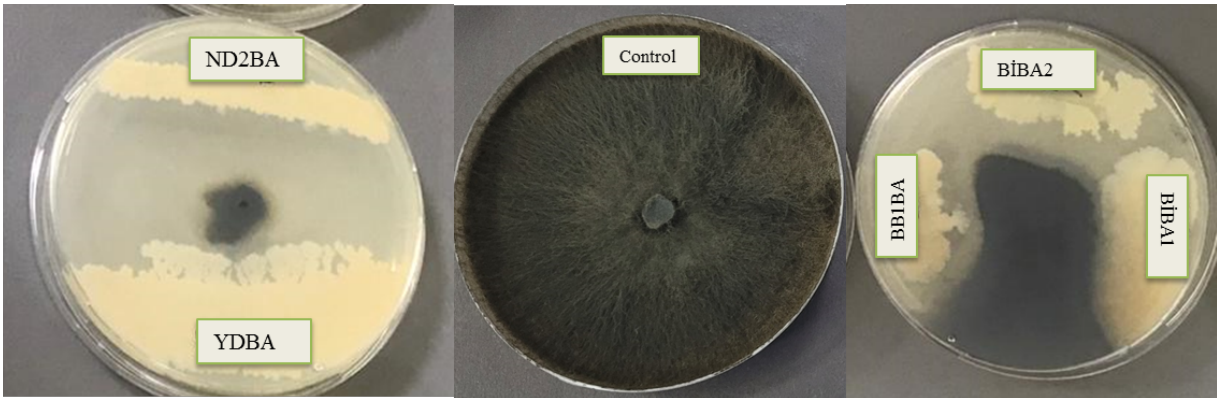

Figure 3. Antagonistic activities of endophytic bacteria against *Neoscytalidium dimidiatum.*

Al Hamad et al. (2021) studied the potential of actinobacterial isolates as biological control agents (BCAs) against the pathogen *N. dimidiatum*. Their research reported the inhibitory ability of *Streptomyces griseorubens* (UAE2) and *Streptomyces wuyuanensis* (UAE1). They reported that these strains exhibited strong antifungal activity by producing antifungal compounds and lytic enzymes. Since this is the first study to study endophytic *Bacillus* sp. against the pathogen *N. dimidiatum*, these studies are important for further studies.

3.3. Applications

According to the one-way analysis of variance (ANOVA) of the obtained observation values, the differences between the averages of the data shown by the root and coleoptile lengths of endophytic bacteria were found to be significant (Table 4).

Table 4. Variance analysis table of root and coleoptile lengths of endophytic bacteria

Coleoptile Length					
Sources of Variation	**DF**	**SS**	**MS**	**F Value**	***P***
Isolates	9	53.791	5.977	60.673	<.0001***
Rep.	3	0.203	0.068	0.686	0.568
Error	27	2.660	0.099		
General	39	56.654			
Root Length					
Sources of Variation	**DF**	**SS**	**MS**	**F Value**	***P***
Isolates	9	81.180	9.020	84.894	<.0001***
Rep.	3	0.159	0.053	0.498	0.687
Error	27	2.869	0.106		
General	39	84.208			

DF: Degrees of freedom SS: Sum of squares MS: Mean of squares.
*** Difference between bacteria according to Tukey LSD Significant according to α = 0.05.

Table 5. Variance analysis table of root and coleoptile lengths of wheat inoculated with endophytic bacteria and *Fusarium culmorum* pathogen

Coleoptile Length					
Sources of Variation	**DF**	**SS**	**MS**	**F Value**	***P***
Isolates	9	87.336	9.704	154.236	<.0001***
Rep.	3	0.439	0.146	2.325	0.097
Error	27	1.699	0.063		
General	39	89.473			
Root Length					
Sources of Variation	**DF**	**SS**	**MS**	**F Value**	***P***
Isolates	9	78.007	8.667	178.251	<.0001***
Rep.	3	0.106	0.035	0.728	0.544
Error	27	1.313	0.049		
General	39	79.426			

DF: Degrees of freedom SS: Sum of squares MS: Mean of squares.
*** Difference between bacteria according to Tukey LSD Significant according to α = 0.05.

As a result of the applications, the root and coleoptile lengths of wheat inoculated with endophytic bacteria were classified as short, medium, medium-long, and long. Coleoptile class values were given to classes with 30-40 mm coleoptile lengths as short, 41-51 mm as medium-short, 52-62 mm as medium, 63-73 mm as medium, and 74-84 mm as long (Table 6). In the application of only bacteria, the BMBA2 isolate with a coleoptile length of 76 mm was classified as long, while the control was classified as short, with a length of 35 mm (Table 6).

Root lengths of wheat inoculated with endophytic bacteria were evaluated as short for those with 30-40 mm root lengths, 41-51 mm as medium short, 52-62 mm as medium, 63-73 mm as medium long, and 74-84 mm as long (Table 6). Only in the bacterial application, the BMBA2 isolate, which had a root length of 83 mm from wheat, was classified as long, while the control was classified as short, with a length of 31 mm (Table 6). In the study conducted by Çelikten and Bozkurt (2018), 69 of the tested isolates caused an increase in root length by 7.1-70.6% compared to the control application. In terms of shoot development, all of the isolates positively increased shoot development and supported our study by increasing shoot length by 6.6-108.6% compared to the control application.

Table 6. Root and coleoptile lengths of plants treated with endophytic bacteria

Isolates	Coleoptile Length (cm)	Root Length (cm)
Control	3.50^{e}	3.05^{g}
BiBA1	4.58^{cd}	4.30^{f}
BiBA2	4.20^{de}	4.73^{ef}
NDBA	5.48^{b}	4.90^{ef}
ND2BA	5.58^{b}	5.25^{de}
ND3BA	5.78^{b}	6.38^{c}
YDBA	7.08^{a}	7.18^{b}
BMBA1	5.35^{b}	5.05^{def}
BMBA2	7.58^{a}	8.33^{a}
BMBA3	5.28^{bc}	5.78^{cd}
Mean	5.44	5.49
SE	0.157	0.163

There is no statistically significant difference between values.

The coleoptile classes of wheat inoculated with endophytic bacteria and *F. culmorum* pathogen were evaluated as short for classes of 20-30 mm, medium short for classes of 31-41 mm, medium for classes of 42-52 mm, medium long for classes of 53-63 mm, and long for classes of 64-74 mm (Table 7). In the application of only bacteria, the BMBA2 isolate had a coleoptile length of 69.8 mm from wheat and was classified as long, while the control was classified as short, with a length of 23.8 mm (Table 7).

The root lengths of wheat inoculated with endophytic bacteria and *F. culmorum* pathogen were evaluated as short for classes of 19-29 mm, medium short for classes of 30-40 mm, medium long for classes of 41-51 mm, medium long for classes of 52-62 mm, and long for classes of 63-73 mm (Table 7). In the bacterial application only, the BMBA2 isolate with a root length of 73 mm was classified as long and the control was classified as short with a root length of 21.5 mm (Table 7).

Table 7. Root and coleoptile lengths of wheat inoculated with endophytic bacteria and *Fusarium culmorum* pathogen

Isolates	Coleoptile Length (cm)	Root Length (cm)
Fusarium culmorum	2.38^{f}	2.15^{e}
BiBA1	3.45^{de}	3.13^{d}
BiBA2	2.85^{ef}	3.08^{d}
NDBA	4.53^{c}	4.13^{c}
ND2BA	3.10^{de}	3.48^{d}
ND3BA	5.80^{b}	4.23^{c}
YDBA	6.23^{b}	5.40^{b}
BMBA1	4.58^{c}	4.88^{b}
BMBA2	6.98^{a}	7.30^{a}
BMBA3	3.50^{d}	3.23^{d}
Mean	4.34	4.098
SE	0.13	0.11

There is no statistically significant difference between values.

Many studies are showing that bacteria that promote plant growth *in vitro* increase seed germination, root and shoot development in many plants such as wheat, corn, and sunflower, and our results are supported by these studies (Mishra et al., 2010; Öksel et al., 2022).

3.4. Determination of metabolic enzyme profiles of isolates with Bruker Maldi Biotyper

Nine isolates from the bacteria to be tested were selected by looking at their BiBA1, BiBA2, NDBA, ND2BA, ND3BA, and YDBA. BMBA1, BMBA2, and BMBA3 enzyme activities, carbohydrate test, and antagonistic activities, and were performed with a matrix-assisted laser desorption ionization-time-of-flight mass spectroscopy (MALDI-TOF MS) device (Bruker Microfleks LT Biotyper. Bruker Daltonics. Bremen. Germany).

As a result of in vitro studies with a matrix-assisted laser desorption ionization-time-of-flight mass spectroscopy (MALDI-TOF MS) device, it was determined that the BİBA1 and ND3BA isolates found to be effective were *B. amyloliquefaciens* subsp. *plantarum*. NDBA, ND2BA, BMBA1. It was determined that BMBA2 and BMBA3 isolates were *B. mojavensis*; BiBA2 and YDBA isolates were *B. subtilis* (Table 8). The fact that the most studied and used biological agent against various plant pathogens is Bacillus species is also supported by numerous studies on *B. subtilis, B. velezensis,* and *B. amyloliquefaciens* species (Shafi et al., 2017; Fira et al., 2018; Caulier et al., 2019).

Table 8. Species identification through metabolic enzyme profiles of isolates with Bruker Maldi Biotyper

Isolates	Isolated plant	Species
BiBA1	Rosemary (*Rosmarinus officinalis* L.)	*Bacillus amyloliquefaciens* subsp. *plantarum*
BiBA2	Rosemary (*Rosmarinus officinalis* L.)	*Bacillus subtilis*
NDBA	Olive (*Olea europaea* L.)	*Bacillus mojavensis*
ND2BA	Olive (*Olea europaea* L.)	*Bacillus mojavensis*
ND3BA	Olive (*Olea europaea* L.)	*Bacillus amyloliquefaciens* subsp. *plantarum*
YDBA	Loquat (*Eriobotrya japonica* (Thunb.) Lindl.)	*Bacillus subtilis*
BMBA1	Olive (*Olea europaea* L.)	*Bacillus mojavensis*
BMBA2	Olive (*Olea europaea* L.)	*Bacillus mojavensis*
BMBA3	Olive (*Olea europaea* L.)	*Bacillus mojavensis*

There are numerous reports on endophytic *Bacillus* strains used for the control of fungal pathogens in different crops. Pan et al. (2015) reported that endophytic *B. megaterium* and *B. subtilis* obtained from wheat grain inhibited the fungal growth of *F. graminearum*. In other studies, some strains of endophytic *B. mojavensis* were reported to protect plants against diseases (Bacon and Hinton, 1996 and 2007; Bacon et al., 2001).

4. Conclusion

In this study, three species of endophytic bacteria belonging to the genus *Bacillus* that were found effective were studied for their antagonistic activity against wheat root and coleoptile lengths and the *F. culmorum* pathogen. *Bacillus mojavensis* (BMBA2) isolates gave the most effective result in coleoptile and root length, followed by *B. subtilis* (YDBA) and *B. amyloliquefaciens* subsp. *plantarum* (ND3BA). This study emphasizes the importance of preparing bioinoculants of selected isolates that show antagonistic activity against pathogens that promote plant growth. *In vitro* conditions, nine potential isolates (BiBA1, ND3BA, NDBA, ND2BA, BMBA1, BMBA2, BMBA3, BiBA2, and YDBA) were determined to be antagonistic against fungal pathogens of endophytes that promote seed root and coleoptile growth in seed germination period characteristics. The results confirmed that endophytes have potential inoculant properties for effective colonization and use as antagonists against pathogens.

It is thought that it may have promising applications in terms of obtaining effective results in the parameters during the wheat seed germination period. Investigating the potential of environmentally friendly biological control agents and environmentally friendly biopesticides in controlling *N. dimidiatum* and *F. culmorum* pathogens may offer sustainable alternatives for disease management. It is also thought that it will contribute to the development of effective strategies for disease management and reduction of the *N. dimidiatum* pathogen.

Ethical Statement

Ethical approval was not required for this study, as the methods employed did not necessitate review by an ethics committee.

Conflict

The author declares that there are no conflicts of interest.

Funding Statement

The author declares that this study was self-funded and received no external financial support.

Author Contributions

The author takes sole responsibility for the conception, design, data collection, analysis, interpretation, and writing of this manuscript.

Acknowledgements

The author acknowledges the support provided by the Department of Plant Protection, Faculty of Kızıltepe Agricultural Sciences and Technologies, Mardin Artuklu University.

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