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Original article (Orijinal araştırma)

Biological control potential of some selected endophytic bacteria against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae), and their effects on potato plant growth

Bazı endofitik bakterilerin patates böceği, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae)'ya karşı biyolojik mücadele potansiyeli ve bitki büyümesi üzerindeki etkileri

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Abstract

This study evaluated the efficacy of six selected endophytic bacteria both against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae), and potential impact on potato plant growth. The bacterial isolates, selected based on prior activities, were tested in *in vivo* pot trials against *L. decemlineata* larvae. Their influence on plant growth was also measured under both pest-infested and pest-free conditions. The experiments were conducted between 2022 and 2024 under controlled growth chamber conditions at the Plant Protection Department of Ege University's Faculty of Agriculture. Bacterial applications caused corrected larval mortality rates of 21.87-56.25% compared to the untreated control. The most effective isolate, *Chryseobacterium gleum* (Holmes et al.) Vandamme et al. (Flavobacteriales: Weeksellaceae) (6), caused 56.25% mortality and exhibited significantly high proteolytic activity. These results demonstrate that the selected endophytic bacteria can significantly reduce *L. decemlineata* populations and enhance plant growth, showcasing their potential as dual-action biocontrol agents in potato cultivation.

Keywords: Biological control, endophytic bacteria, *Leptinotarsa decemlineata,* plant growth promoters, potato beetle

Öz

Bu çalışma, seçilen altı endofitik bakterinin Colorado patates böceği, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) üzerindeki etkinliğini ve patates bitkisinin büyümesi üzerindeki potansiyel etkisini değerlendirmiştir. Önceki faaliyetlere göre seçilen bakteri izolatları, *L. decemlineata* larvalarına karşı *in vivo* saksı denemelerinde test edilmiştir. Bitki büyümesi üzerindeki etkileri de hem zararlının bulunduğu hem de zararlının bulunmadığı koşullarda ölçülmüştür. Denemeler, 2022 ile 2024 yılları arasında Ege Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü'ne ait kontrollü büyüme odası koşullarında gerçekleştirilmiştir. Bakteriyel uygulamalar, uygulama görmemiş kontrol grubuna kıyasla %21.87-56.25 oranında larva ölüm oranlarına neden olmuştur. En etkili izolat olan *Chryseobacterium gleum* (Holmes et al.) Vandamme et al. (Flavobacteriales: Weeksellaceae) (6), %56.25 ölüm oranına neden olmuş ve önemli ölçüde yüksek proteolitik aktivite göstermiştir. Bu sonuçlar, seçilen endofitik bakterilerin *L. decemlineata* popülasyonlarını önemli ölçüde azaltabildiğini ve bitki büyümesini artırabildiğini göstererek, patates yetiştiriciliğinde çift yönlü etkili biyolojik mücadele ajanları olarak potansiyellerini ortaya koymaktadır.

Anahtar kelimeler: Biyolojik mücadele, endofitik bakteriler, *Leptinotarsa decemlineata*, bitki büyüme teşvik edicileri, patates böceği

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Introduction

The potato, *Solanum tuberosum* L. (Solanales: Solanaceae) plays a significant role in human nutrition due to its carbohydrate, protein, mineral, and vitamin contents. It is cultivated in nearly all countries across diverse climatic conditions. The Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae), is the most prevalent and destructive pest associated with this crop, resulting in substantial economic losses, particularly in potato production (Hare, 1990; Weber, 2003; Stieha & Poveda, 2015; Alyokhin et al., 2022). Both the larval and adult stages inflict damage on the plant. This insect exhibits voracious feeding behavior, capable of consuming up to 40 cm² of potato foliage during its larval stage and approximately 10 cm² per day as an adult. In the absence of effective control measures, this pest can lead to significant reductions in tuber yield and quality, particularly in terms of tuber size (Alyokhin et al., 2022).

The management of *L. decemlineata* predominantly relies on the application of chemical insecticides. Nonetheless, many of the chemicals utilized are environmentally toxic, adversely affect non-target organisms, and can leave residues within food products. Furthermore, the pest's development of resistance presents a significant challenge (Grafius, 1997; Alyokhin et al., 2008; Szendrei et al., 2012; Scott et al., 2015; Kuhar et al., 2022; Chen et al., 2023; Kocourek et al., 2024). For instance, *L. decemlineata* has developed resistance to 57 distinct compounds across all major classes of insecticides (APRD, 2025). Moreover, the rates of insecticide resistance evolution in *L. decemlineata* are among the highest documented in agricultural pests (Brevik et al., 2018; Brevik et al., 2021). Therefore, it is imperative to develop safe alternative control strategies to mitigate further resistance development in *L. decemlineata*.

Biological control represents a promising approach for pest management. In particular, the use of microbial agents, particularly entomopathogens, is increasingly recognized as a viable solution to the challenges posed by pest resistance. Specifically, research on biological control using entomopathogenic bacteria, offers environmentally sustainable alternatives that have demonstrated efficacy against various pest populations. A promissing subgroup of these microorganisms are endophytic bacteria, which can colonize internal plant tissues without causing disease and confer benefits such as enhanced plant growth, pathogen supression, and pest control (Sgroy et al., 2009).

Positive outcomes have been observed from endophytes in research addressing various fungal and bacterial pathogens affecting both annual and perennial plant species (Lodewyckx et al., 2002; Bargabus et al., 2004; Kloepper et al., 2004; Andreote et al., 2009; Ramesh & Phadke, 2012; Lanna-Filho et al., 2013). Furthermore, in recent years, endophytic bacteria have been demonstrated to be effective biological control agents against various insect pests (Azevedo et al., 2000).

Eighteen bacterial species exhibiting antimicrobial activity were isolated from the gut microbiota of *L. decemlineata* adults and larvae, as well as from their diet (potato leaves). This finding supports the proposition that the gut microbiota of *L. decemlineata* is a promising source of biopesticide components (Kang et al., 2021).

Serratia marcescens J. (Enterobacterales: Enterobacteriaceae) is a promising bacterial agent in the biological control of plant pathogens and insect pests. It achieves various mechanisms, including the production of antimicrobial metabolites and the induction of systemic resistance in plants (Akhtar et al., 2025).

One investigation of microbial communities for biological control agents against *L. decemlineata* identified several bacteria, including *Leclercia adecarboxylata* C. (Ld1) (Enterobacterales: Enterobacteriaceae), *Acinetobacter* sp. (Ld2, Ld3, Ld5), *Acinetobacter haemolyticus* Bouvet and Grimont (Ld6) (Pseudomonadales: Moraxellaceae) and *Pseudomonas putida* Migula (Ld4) (Pseudomonadales: Pseudomonadaceae). When tested at a concentration of 1.8×10^9 cfu/ml, *L. adecarboxylata* (Ld1) and *P. putida* (Ld4) caused the highest larval mortality. These findings suggest that these two bacterial strains may serve as promising candidates for biological control strategies targeting *L. decemlineata* (Muratoğlu et al., 2011).

Endophytic bacteria can promote plant growth directly through the production of phytohormones, such as indole-3-acetic acid (IAA), and indirectly by modulating the synthesis of plant hormones within the host (Lazarovits & Nowak, 1997; Lee et al., 2004).

Endophytic bacteria serve numerous critical and beneficial functions in their host plants, including biological nitrogen fixation, siderophore-mediated iron acquisition, phosphate solubilization, the synthesis of plant growth hormones, a reduction in stress ethylene levels through the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, the degradation of toxic compounds, and the inhibition of fungal and bacterial pathogens. Moreover, internal plant tissues create a protective environment that facilitates colonization by endophytic bacteria, a niche similarly exploited by pathogenic organisms.

In recent years, the utilization of endophytic bacteria for management of plant pathogens and insect pests has emerged as a more sustainable alternative to synthetic pesticides. Indeed, recent studies highlighted that biofertilizers and biopesticides are playing a major role in reducing dependence on chemical inputs and promoting environmentally sustainable agricultural practices (Hallmann et al., 1998).

Building on this foundation, an investigation involving 19 distinct endophytic bacteria isolates against *L. decemlineata* larvae under controlled laboratory conditions, found that some isolates caused significant mortality rates (Özsarı et al., 2017). The present study aims to further explore the efficacy of these promissing bacterial endophytes, previously identified as effective in the biological control of *L. decemlineata* in *in vitro* settings, as entomopathogens through *in planta* pot trials. Given that these isolates possess entomopathogenic properties against *L. decemlineata*, as well as potential to enhance plant growth and suppress diseases. This study will also assess their influence on plant growth under both pest-free conditions and under stress induced by *L. decemlineata*.

Materials and Methods

Study site and environmental conditions

All experiments were conducted under controlled growth chamber conditions, maintained at a temperature of 25±1ºC, a relative humidity of 55±5%, and a photoperiod comprising 16 hours of light and 8 hours of darkness. These experiments took place at Ege University, Faculty of Agriculture, Department of Plant Protection, during the period from 2022 to 2024. Furthermore, the rearing of insects and the cultivation of potato plants were performed under identical environmental conditions (Doležal et al., 2007).

Establishment of a rearing colony of *Leptinotarsa decemlineata*

Adult *L. decemlineata* specimens were collected from potato fields in Bozdağ (Izmir Province, Türkiye) and subsequently transported to the laboratory. A stock colony of *L. decemlineata* was established and maintained over three generations. Insects derived from this stock colony were utilized in the experimental procedures. Wooden and thin-mesh cages (43 × 75 × 65 cm) were employed for the rearing of the insects. Within these cages, potato plants of the Marabel variety, approximately 8-10 leaves in number and 18-20 cm in height, were provided as a food source and oviposition substrate. Insect rearing and cultivation of potato plants were conducted under uniform growth chamber conditions. A long-day photoperiod (16: 8 Light: Dark) was implemented to inhibit adults from entering diapause. Food plants were replaced every 48 h to ensure continuous fresh foliage availability. Egg masses laid under potato leaves were collected daily and transferred to clean cages, and newly hatched neonates were synchronized to obtain uniform second-instar larvae for bioassays.

Production of the endophytic bacteria used in the study

In a prior investigation, the biological efficacy of five endophytic bacteria isolates (6, 53, 83, 99, and 103) was assessed, revealing that these isolates induced over 30% mortality across all larval stages of *L. decemlineata* in a detached leaf assay conducted under laboratory conditions (Özsarı et al., 2017). The characteristics of these isolates, as detailed in Table 1, were further examined in an *in planta* pot experiment involving *L. decemlineata* larvae within a controlled plant growth chamber environment. Additionally, a bacterial isolate, designated 443, was included. This isolate was originally isolated from blackened *L. decemlineata* larvae collected during field surveys and was purified using standard protocols for endophytic bacterial isolation (Hallmann et al., 1998). Its pathogenicity was confirmed by fulfilling Koch's postulates, thereby verifying its entomopathogenic nature. All isolates of Chitinase activities were determined by measuring the width of the clear zone formed around bacterial colonies 10 days after spot inoculation, following the methods described by Chernin et al. (1995) and Wang et al. (2013). Protease activities were assessed 4 days after spot inoculation on Luria Bertani agar medium amended

with 3% skim milk, as described by Khabbaz et al. (2015). The bacterial isolate 443 was molecularly characterized using 16S rRNA gene sequencing and identified as *S. marce*scens (Table 1). All six isolates were retrieved from a deep freezer maintained at -80ºC and subsequently streaked onto King's B (KB) medium (King et al., 1954), allowing for growth in an incubator at 24ºC for a duration of 24 to 48 hours. All six bacterial isolates were retrieved from −80ºC glycerol stocks and streaked onto King's B (KB) agar medium (King et al., 1954). Plates were incubated at 24ºC for 24-48 h. A single colony from each isolate was transferred into 50 mL of Nutrient Broth (NB) and incubated at 24ºC with shaking at 180 rpm until reaching the exponential growth phase. Bacterial cells were harvested and resuspended in sterile distilled water supplemented with 0.1% Tween 20. Optical density measurements at 600 nm ($OD_{600} \approx 0.1$) were used only as a preliminary reference. The final concentration of each bacterial suspension was empirically adjusted to 1×10^8 cfu/ml based on serial dilution and plate counting, which was performed separately for all isolates prior to bioassays.

Table 1. Information on the origin, entomopathogenic properties and diagnosis of the bacteria used in this study (Özsarı et al., 2017)

Origin			Entomopathogenic properties			Molecular identification			
No[a]	Code[b]	Host[c]	Mort[*d]	CA[e]	PA[f]	Species	RSR[g]	RAN[h]	NAN[i]
6[x]	CB2/3	CS[j]	70	0.00	13.75	*C. gleum*[l]	99.00%	NCTC11432	LR134289.1
53[x]	CC27	CS	65	0.00	7.75	*P. baetica*[m]	99.81%	KY939741.1	PV856402
83[x]	CC37/2	CS	65	0.00	1.00	*P. agglomerans*[n]	99.00%	NR_041978.1	KX589255
99[x]	CA41/1	CS	30	7.00	2.00	*B. thuringiensis*[o]	99.93%	KJ009430.1	PV810205
103[x]	CC41/2	CS	65	0.00	0.00	*B. brevis*[p]	99.79%	NR_041978.1	PV856403
443[y]	LD443	CPB[k]	50	10.75	9.75	*S. marcescens*	98.26%	KC335216.1	PV991438

a No: Isolate No; b Code: Isolate Code; c Host: Isolated Host; d Mort: Average mortality rate for all larval stages; e CA: Chitinase Activity (mm)**; f PA: Protease Activity (mm)**; g RSR: Reference Similarity Rate; h RAN: Reference accession number; i NAN: NCBI Access No; j CS: *Cucumis sativus* L. (Cucurbitales: Cucurbitaceae)*;* k CPB: Colorado Potato Beetle; l *C. gleum*: *Chryseobacterium gleum* (Holmes et al.) (Flavobacteriales: Weeksellaceae); m *P. baetica*: *Pseudomonas baetica* López et al. (Pseudomonadales: Pseudomonadaceae); n *P. agglomerans*: *Pantoea agglomerans* (Beijerinck) (Enterobacterales: Enterobacteriaceae); o *B. thuringiensis*: *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae); p *B. brevis*: *Brevibacillus brevis* (Migula) (Brevibacillales: Brevibacillaceae); x: Isolates obtained in Özsarı et al., 2017; y: isolate obtained from blackened *L. decemlineata* larvae in 2024 (unpublished data).

Effect of endophytic bacteria on *Leptinotarsa decemlineata* mortality and its feeding damage on potato plants

Potato tubers (*Solanum tuberosum* cv. Marabel) were cultivated in 1.5-liter cylindrical plastic pots, which measured 17 cm in diameter and 14 cm in height, filled with sterile peat. At the 8-10 leaf stage, a suspension of endophytic bacteria isolates at 10^9 cfu/ml in a 0.1% Tween 20 solution was applied to the shoots, with an application volume of 20 ml per plant. Control plants were sprayed with sterile distilled water containing 0.1% Tween 20. The negative control treatment excluded *L. decemlineata* larvae, while the positive control treatment included the larvae. Bacterial suspensions were applied using a hand-held manual sprayer, ensuring uniform coverage of both adaxial and abaxial leaf surfaces until run-off. Tween 20 (0.1%) was used as the sole surfactant, and no additional wetting agents were applied. Each application was set up according to a randomized plot design with eight replicates. Each replicate consisted of a single plant and four second-instar potato beetle larvae. Second-instar larvae were introduced onto treated plants 24 h after bacterial application to allow bacterial establishment on plant surfaces. For the bioassays, only second-instar larvae of *L. decemlineata* were used. All larvae were of similar developmental stage at the beginning of the experiment. No separate bioassays were conducted using different larval instars. Mortality recorded at the pupal and adult stages reflects the progression of initially treated larvae through subsequent developmental stages during the experimental period. Plant-insect interactions were assessed over a period of 20 days, during which adult emergence was recorded in all replicates. To evaluate the entomopathogenic effects of endophytic bacteria on *L. decemlineata* larvae under *in planta* conditions, mortality rates were analyzed. Larval mortality was assessed daily and recorded when individuals showed no movement after gentle probing with a fine brush. Mortality rates were calculated by monitoring the same cohort of larvae throughout the experimental period. Larval, pupal, and adult mortality values reflect stage-specific mortality recorded as the initially treated second-instar larvae progressed through subsequent developmental stages. In addition, at the end of the 20-day observation period, the root and shoot parts were weighed separately and dried at 70±1ºC for 48 hours to evaluate the feeding status of *L. decemlineata* larvae feeding on endophytic bacteria-treated potato plants. Feeding status was indirectly assessed based on final root

and shoot dry weight, as a proxy for cumulative larval feeding pressure. Direct leaf area consumption measurements were not performed. Furthermore, at the conclusion of the 20-day observation period, the root and shoot components of the plants were weighed separately and subjected to drying at a controlled temperature of 70±1ºC for a duration of 48 hours. This procedure was used to evaluate the feeding status of *L. decemlineata* larvae that consumed endophytic bacteria-treated potato plants. Consequently, the impact of endophytic bacteria treatments on plant growth, despite the presence of *L. decemlineata* larvae, was systematically evaluated.

Plant growth promotion assay in the absence of *Leptinotarsa decemlineata*

The plant growth-promoting effects of the endophytic bacteria were assessed separately in the absence of insect larvae. A suspension of endophytic bacteria (10^9 cfu/ml, 20 ml per plant) was applied to potato plants as a foliar application at the 8 to 10 leaf stage. Control plants were sprayed with a solution of sterile distilled water containing 0.1% Tween 20. The impact of endophytic bacteria on plant growth was measured at the conclusion of a 20-day observation period by quantifying the dry weight of both the root and shoot parts of the plants. These experiments were conducted with eight replicates, with one plant per replicate. The shoot and root parts were weighed separately and subsequently dried at 70±1ºC for 48 hours.

Data analysis

Prior to analysis, data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test prior to analysis. Following confirmation of these assumptions, treatment effects were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected, mean comparisons were performed using Tukey HSD test at a 95% confidence level (p<0.05). All statistical analyses were conducted using IBM SPSS Statistics 24.0.

Results

Effect of endophytic bacteria on the cumulative mortality rate of *Leptinotarsa decemlineata*

The cumulative mortality of *L. decemlineata* across its life stages following treatment with endophytic bacteria is presented in Table 2 and Figure 2.

Table 2. Cumulative mortality (%) of *Leptinotarsa decemlineata* across its life stages following treatment with endophytic bacteria at the second larval instar on potato plants (Mean±SE)

Endophytic bacteria isolate number/species	Number of second-instar larvae tested	Cumulative mortality (%)*
6 – *Chryseobacterium gleum*	32	56.25±8.41 b
53 – *Pseudomonas baetica*	32	28.12±7.24 ab
83 – *Pantoea agglomerans*	32	28.12±8.15 ab
99 – *Bacillus thuringiensis*	32	21.87±2.99 ab
103 – *Brevibacillus brevis*	32	50.00±9.01 b
443 – *Serratia marcescens*	32	43.75±3.18 b
Control	32	0.00±0.00 a

* Different letters in a column indicate a significant difference between the treatments by Tukey HSD Multiple Range Test (p<0.05).

According to one-way ANOVA followed by Tukey's HSD test, significant differences in mortality were detected between the untreated control and treatments with *C. gleum* (6), *B. brevis* (103), and *S. marcescens* (443) ($F(6, 49)=3.96$; p=0.0026). In contrast, mortality rates observed in treatments with *B. thuringiensis* (99), as well as strains 53 and 83, did not differ significantly from the control. Among the tested endophytic bacteria, *C. gleum* (6) was the most effective treatment, inducing the highest average mortality (56.25%), followed by *B. brevis* (103) (50.00%) and *S. marcescens* (443) (43.75%) (Table 2).

Figure 1. *In vivo* test results: In the photos show dead larvae following treatment with a) *Serratia marcescens* (443) and b) *Chryseobacterium gleum* (6), c) as well as a healthy larva, verified in the control treatment.

When evaluating the effects of endophytic bacterial treatments on different developmental stages of *L. decemlineata*, mortality was predominantly observed during the larval stage (Figure1), particularly in plants treated with *C. gleum* (6) and *B. brevis* (103), followed by *P. agglomerans* (83). All treatments except *P. baetica* (53) caused larval mortality. *P. baetica* (53) did not induce larval mortality but caused mortality exclusively at the pupal stage. Overall, *C. gleum* (6) was the only isolate that resulted in mortality across all developmental stages, including larvae, pupae, and adults (Figure 2).

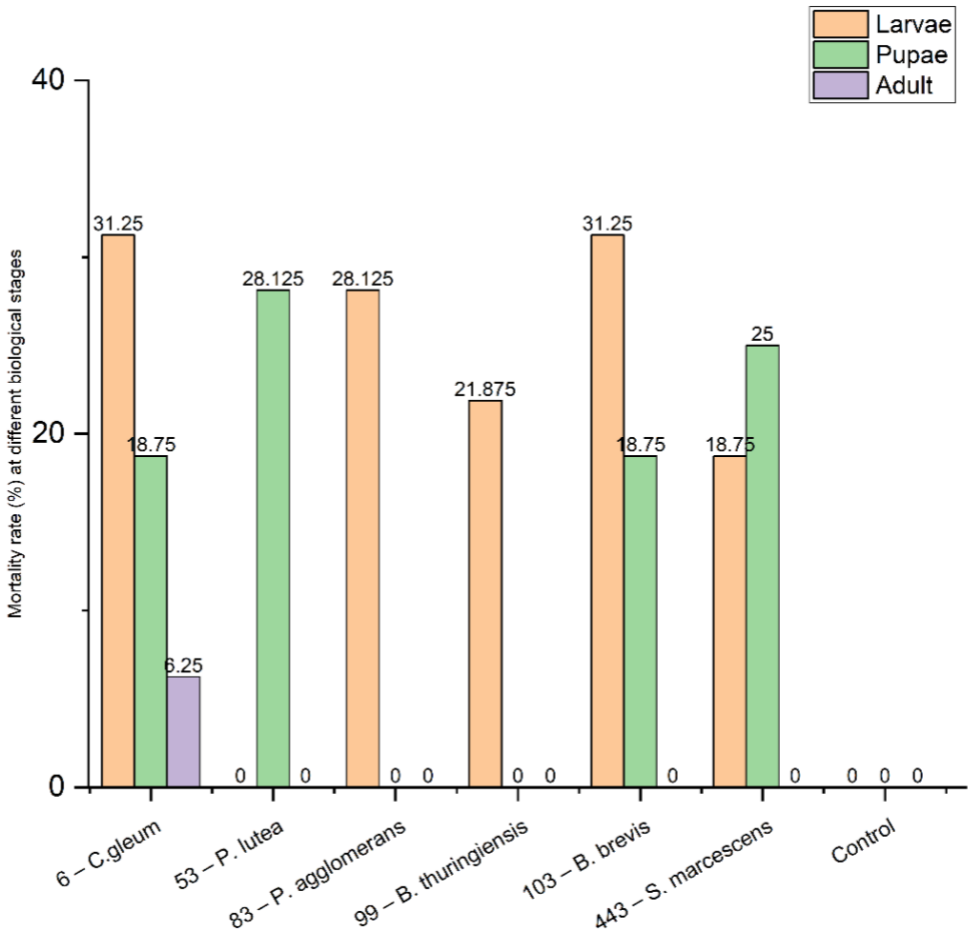

Figure 2. Mortality rate of *Leptinotarsa decemlineata* subjected to endophytic bacteria treatments at different biological stages of development: larvae, pupae, and adults.

Effect of endophytic bacteria on the feeding status of *Leptinotarsa decemlineata* in potato plants

The feeding behavior of *L. decemlineata* on potato plants subjected to endophytic bacteria treatment was observed over a period of 20 days and subsequently compared to that of untreated control plants.

One-way analysis of variance (ANOVA) revealed statistically significant treatment effects on plant growth parameters during *L. decemlineata* feeding ($F(6,49)=9.27$; $p<0.001$). Based on shoot dry weight measurements, plants treated with isolates 6, 53, and 443 showed significantly higher shoot biomass than the control, with mean values ranging from 92.13 to 93.25 g plant^{-1}, whereas the control plants recorded a shoot dry weight of 86.28 g plant^{-1}. In contrast, shoot dry weights of plants treated with isolates 83 and 99 did not differ significantly from the control (Table 3).

With respect to root development, significant differences were detected among treatments ($F(6,49)=14.13$; $p<0.001$). Root dry weight values of endophytic bacteria–treated plants ranged from 77.40 to 91.24 g plant^{-1}, while the control plants exhibited the lowest root biomass (72.32 g plant^{-1}). Among the tested isolates, 83 and 99 resulted in the highest root dry weight values and were placed in the same statistical group (Table 3).

Table 3. Effect of endophytic bacteria treatments on potato plant feeding of *Leptinotarsa decemlineata* (Mean±SE)

Endophytic bacteria isolate no / species	Average dry weight of shoots (g/plant)	Average dry weight of roots (g/plant)
6 – *Chryseobacterium gleum*	92.13±0.89 a*	77.40±2.41 bc*
53 – *Pseudomonas baetica*	93.25±0.54 a	80.60±2.42 b
83 – *Pantoea agglomerans*	87.14±1.12 b	91.24±0.46 a
99 – *Bacillus thuringiensis*	86.33±0.83 b	90.70±0.65 a
103 – *Brevibacillus brevis*	89.16±1.48 ab	77.95±2.62 bc
443 – *Serratia marcescens*	92.70±0.66 a	80.12±1.16 b
Control	86.28±1.17 b	72.32±2.21 c

* According to Tukey HSD multiple comparison test, the difference between the means indicated by the same letters in the same column is not significant according to the $p<0.05$ value.

Effects of endophytic bacteria applications on plant growth

The effects of endophytic bacteria on plant growth in the absence of *L. decemlineata* larvae are presented in Table 4. With respect to shoot dry weight, all endophytic bacterial isolates showed statistically significant increases compared with the untreated control ($F(6,49)=17.23$; $p<0.001$). Plants treated with endophytic bacteria exhibited higher shoot dry weight values, ranging from 88.93 to 91.20 g plant^{-1}, whereas the control plants recorded the lowest shoot biomass (64.28 g plant^{-1}).

Table 4. Effect of endophytic bacteria treatments on root and shoot dry weight of potato plants without *Leptinotarsa decemlineata* (Mean±SE)

Endophytic bacteria isolate no / species	Average dry weight of shoots (g/plant)*	Average dry weight of roots (g/plant)
6 – *Chryseobacterium gleum*	90.15±0.77 a*	78.89±1.82 b*
53 – *Pseudomonas baetica*	89.62±0.70 a	80.70±1.43 b
83 – *Pantoea agglomerans*	88.93±0.93 a	90.64±0.21 a
99 – *Bacillus thuringiensis*	89.62±0.70 a	89.73±1.74 ab
103 – *Brevibacillus brevis*	89.65±0.54 a	75.72±2.63 b
443 – *Serratia marcescens*	91.20±1.06 a	78.08±2.34 b
Control	64.28±9.29 b	69.87±4.29 c

* According to Tukey HSD's multiple comparison test, the difference between the means indicated by the same letters in the same column is not significant according to the $p<0.05$ value.

Regarding root development, all endophytic bacterial treatments resulted in significantly higher root dry weight values than the untreated control ($F(6,49)=20.92$; $p<0.001$). Root dry weight among treated plants ranged from 75.72 to 90.64 g plant^{-1}, while the control plants showed the lowest root biomass (69.87 g plant^{-1}). Among the tested isolates, isolate 83 produced the highest root dry weight, followed by isolate 99, which was placed in an intermediate statistical group (Table 4).

Discussion

Alternative solutions should be developed due to the polyphagous nature of *L. decemlineata*, its rapid development of resistance to chemical insecticides, and the associated risks of chemical residues and environmental pollution. This study investigates the biocontrol potential of some endophytic bacteria isolates against *L. decemlineata*, building on findings from a previous *in vitro* bioassay conducted using the detached leaf test (Özsarı et al., 2017). The current research was carried out under *in vivo* conditions through a pot experiment conducted in a plant growth chamber.

Endophytic bacterial isolates are recognized for their entomopathogenic effects on *L. decemlineata*, as well as their potential to promote plant growth and enhance biological control against diseases. The observation that endophytic bacteria selected based on *in vitro* test results (Özsarı et al., 2017) induced an average mortality rate of 22-56% in *L. decemlineata* within this study demonstrates that the *in vivo* test outcomes corroborate the *in vitro* findings. Although all bacterial isolates resulted in numerically higher mortality rates than the control, the effects of *B. thuringiensis* (99), *P. baetica* (53), and *P. agglomerans* (83) were not statistically significant ($p>0.05$). This suggests that while these endophytes possess entomopathogenic potential, their efficacy in a pot trial environment may vary compared to previously conducted *in vitro* assays. These results suggest that the detached leaf assay has been validated as a rapid, efficient, and reliable method for the identification of entomopathogenic candidate endophytic bacterial isolates (Otsu et al., 2004).

In this study, the most effective isolate, causing significant mortality across all developmental stages of *L. decemlineata* (larvae, pupae, and adults), was *C. gleum* (6). This entomopathogenic bacterial isolate was originally isolated from cucumber plants and has been previously recognized for its capacity to enhance plant growth (Özaktan et al., 2015b). Direct reports on the entomopathogenic effects of *C. gleum* are limited; however, a study by Page et al. (2019) indicated that *Chryseobacterium nematophagum* infects and eliminates parasitic nematodes. This suggests a broader pathogenic potential within the *Chryseobacterium* genus. Additionally, several studies have documented the biocontrol and plant growth-promoting effects of this genus against plant pathogens (Krause et al., 2001; Kim et al., 2012; Bhise et al., 2017). In particular, *C. gleum* has demonstrated plant growth-promoting properties, including the production of indole-3-acetic acid, 1-aminocyclopropane-1-carboxylase deaminase activity, siderophores, ammonia, hydrogen cyanide, cellulase, and protease enzyme activities (Bhise et al., 2017). The significant proteolytic activity exhibited by *C. gleum* (6) in this study, may be a key mechanism underlying its observed entomopathogenicity, while its other documented traits likely contributed to the plant growth enhancement noted in our results.

Pantoea agglomerans, a member of the Enterobacteriaceae family, can be extensively isolated from various environments, including plants, aquatic systems, soil, and both animal and human feces (Delétoile et al., 2009). This species has also been identified in numerous insect taxa from diverse orders (Demir et al., 2012; Sevim et al., 2012). In this study, *P. agglomerans* (83), an endophyte extracted from the healthy cucumber plants (Akbaba & Özaktan, 2018) was treated using potato leaves and demonstrated lethal activity against *L. decemlineata* larvae. The insecticidal properties of *P. agglomerans* have been previously documented in several investigations (Hashimoto, 2002; Bahar & Demirbağ, 2007; Sevim et al., 2012; Dutkiewicz, 2016). Additionally, this isolate has been recognized for its effectiveness against Fusarium wilt [*Fusarium oxysporum* f. sp. *melonis* W.C. Snyder & H.N. Hansen (Hypocreales: Nectriaceae)] and bacterial angular leaf spot disease [*Pseudomonas syringae* pv. *lachrymans* van Hall (Pseudomonadales: Pseudomonadaceae)] in cucumber, along with its plant growth-promoting characteristics (Özaktan et al., 2015a; Akbaba & Özaktan, 2018; Akköprü et al., 2021). These attributes underscore its potential application in biological control strategies targeting both plant diseases and pest management. In the current investigation, *P. agglomerans* (83) was also identified as one of the most effective isolates in enhancing the root dry weight of potato plants in the absence of *L. decemlineata* stress, reinforcing its capacity to promote plant growth. However, it is important to note that the growth-promoting effect of isolate 83 on shoot dry weight was only statistically significant in the absence of pest stress. This indicates that severe herbivory by *L. decemlineata* may limit the plant's ability to fully utilize the growth-promoting benefits of certain endophytic strains. A significant increase in root dry weight is particularly advantageous for potato plants, as it is directly correlated with tuber yield. The combination of this plant growth-promoting trait with its observed entomopathogenicity activity suggests that *P. agglomerans* (83) may be a promissing candidate for use in biological control and integrated pest managementstrategies. Therefore, prior to practical application, further research to confirm its efficacy under field conditions is warranted.

As an endophyte, *B. thuringiensis* strain 15A3 has been documented to exhibit potent antifungal activity against *Rhizoctonia solani* J. G. Kühn (Cantharellales: Ceratobasidiaceae)*, Physalospora pyricola* Nose (Botryosphaeriales: Botryosphaeriaceae)*, Penicillium chrysogenum* Thom (Eurotiales: Aspergillaceae)*,* and *Botrytis cinerea* Pers. Ex Nocca & Balb. (Helotiales: Sclerotiniaceae)*,* as well as potential entomopathogenic effects against insect larvae such as *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Liu et al., 2010). The pronounced chitinase activity of this bacterium suggests its efficacy in inducing plant resistance and facilitating biological control. Studies indicate that strains of *B. thuringiensis* employed in the biological control of plant diseases exhibit robust lipopeptide production, with fengycin being particularly predominant among them (Kim et al., 2004). Fengycin, a lipopeptide, not only demonstrates antifungal activity, particularly against fungal pathogens, but also plays a crucial role as a signaling molecule that activates plant resistance mechanisms against pathogen attacks (Ongena & Jacques, 2008). In the present study, *B. thuringiensis* (99), which exhibited entomopathogenic effects against *L. decemlineata* in *in vivo* tests, was found to be effective against several fungal and bacterial diseases (*Fusarium oxysporum* f. sp. *cucumerinum*, *P. syringae* pv. *lachrymans*) in cucurbits. In plants treated with this *B. thuringiensis* isolate, there was an observed increase in the activation of pathogenesis-related genes (PR1, PR3), which are responsible for chitinase production (Özaktan et al., 2015b). Furthermore, this isolate also resulted in an increase in marketable cucumber yield by over 10% compared to untreated plants, demonstrating its multifaceted nature (Özaktan et al., 2015b). In this study, *B. thuringiensis* (99) enhanced root and shoot growth even in the absence of *L. decemlineata*, confirming the strain's growth-promoting capabilities. The observed entomopathogenicity against *L. decemlineata* reported here aligns well with the high chitinase activity previously detected in this strain. This enzymatic activity, which targets chitin in the insect exoskeleton and fungal cell walls, is a key mechanism for biocontrol. Additionally, a separate molecular study confirmed that the expression of PR1 and PR3, genes responsible for chitinase production by this bacterium, increased in treated plants (Özaktan et al., 2015b). Collectively, these multifunctional attributes suggest that this bacterium holds significant promise for integrated pest management programs.

Pseudomonas baetica (53) is an endophytic bacterium isolated from the inner tissues of healthy cucumber plants, and its biological control potential against *F. oxysporum* f. sp. *cucumerinum* was established in prior research (Özaktan et al., 2015a, b). Numerous studies have demonstrated that *P. baetica* possesses antimicrobial activity against plant pathogenic fungi and exhibits significant biological control potential (Egamberdieva et al., 2020; Derikvand et al., 2023). The efficacy of this bacterium in biological control has been linked to its protease and chitinase enzyme activities. This study provides the first evidence of a notable entomopathogenic effect by *P. baetica* (53) against *L. decemlineata*, as no such activity has been documented in the literature to date. The identification of the entomopathogenic characteristics of *P. baetica* in this research constitutes a novel discovery. The elevated proteolytic enzyme activity of *P. baetica* (53), which resulted in significant mortality among *L. decemlineata* in this study, may contribute to its entomopathogenic capabilities. Additionally, the production of auxin (IAA), a key plant growth regulator, by *P. baetica* has facilitated its classification as a plant growth-promoting bacterium (González et al., 2021). The application of *P. baetica* starin 53 to potato foliage significantly increased the dry weight of both shoot and root biomass in the absence of *L. decemlineata* pressure, demostrating its clear potential to enhance plant growth-promoting bacterium. The observed increases in plant biomass may be indirectly associated with reduced feeding efficiency of *L. decemlineata* on endophytic bacteria-treated plants, suggesting a potential deterrent or adverse effect on larval feeding behavior.

Numerous studies have explored the biocontrol potential of bacteria within the genus *Brevibacillus*. Antibacterial peptides (AMPs) constitute the primary antibacterial compounds produced by *Brevibacillus*. Notably, *B. brevis* has demonstrated antagonistic effects against plant pathogenic fungi (Yang et al., 2023). Research indicates that *B. brevis* exhibits promising antifungal activity against the plant pathogens *Fusarium graminearum* Schwabe (Hypocreales: Nectriaceae) and *B. cinerea* (Kim et al., 2024). In the context of biological control of *Erysiphe necator* Schwein. (Helotiales: Erysiphaceae), the agent *B. brevis* CP-1, at a concentration of 1000 ml/100L, was found to be 91.68% effective in controlling powdery mildew in vineyards (Avan et al., 2023). Furthermore, *Brevibacillus* species are known to produce a variety of metabolites and some strains produce crystals, understanding their potential as antimicrobials, insecticides, and sustainable biological control agents (Smirnova et al., 2023). In the present study, *B. brevis* (103) exhibited entomopathogenic activity against *L. decemlineata*, despite the fact that no chitinolytic and/or proteolytic activities were detected. This suggests that

its pathogenicity is mediated by other mechanisms. Therefore, further investigation into the potential toxins, crystals, and other virulence-associated metabolites produced by this strain is warranted to elucidate the mechanism behind the biocontrol efficacy observed against *L. decemlineata*.

Consistent with other biocontrol organisms, *B. brevis* is recognized for its dual capacity to suppress plant pathogens and enhance plant growth. For example, a study assessing the plant growth-promoting potential of *B. brevis* on cotton seeds demonstrated that it produces indole-3-acetic acid (IAA) and exhibits antifungal activity (Nehra et al., 2016). Our results align with this profile, as *B. brevis* (103) significantly enhanced plant growth compared to untreated control plants in the absence of pest pressure. Therefore, the combination of its entomopathogenic activity against *L. decemlineata* and its plant growth-promoting effects positions *B. brevis* (103) as a highly promissing biopesticide candidate for development as a dual-action agent, functioning as both a biopesticide and a biofertilizer in sustainable crop production systems.

Serratia marcescens is recognized as an opportunistic pathogen affecting a variety of organisms, including insects (Tao et al., 2022). *Serratia marcescens* possesses a broad spectrum of entomopathogenic activity targeting significant agricultural pests (Nawani & Kapadnis, 2001; Merzendorfer & Zimoch, 2003). During the pathogenic process, it is proposed that this bacterium induces intestinal rupture by degrading the exoskeleton of insects, the peritrophic membrane, and the intestinal epithelium through the production of various insecticidal metabolites, including proteases, lipases, and chitinases (Hines et al., 1988; Tao et al., 2022). Consequently, the degradation of chitin or the inhibition of chitin metabolism, coupled with the presence of proteolytic enzyme activity, may contribute to the mortality of agricultural pests. The high levels of chitinase and proteinase enzyme activity observed in *S. marcescens* (443), which demonstrated insecticidal efficacy against *L. decemlineata* in this study, may elucidate the entomopathogenic mode of action of this bacterium.

Moreover, *Serratia* species are recognized as significant agents in biological control due to their production of a variety of secondary metabolites possessing antifungal, antibacterial, and entomopathogenic properties. These metabolites have demonstrated effectiveness against numerous plant pests and pathogens. In addition to their insecticidal capabilities, certain *Serratia* species have been documented to mitigate plant diseases, including *F. oxysporum*, *R. solani*, *Phytophthora parasitica* Dastur (Peronosporales: Peronosporaceae), *Sclerotinia sclerotiorum* (Lib.) (Helotiales: Sclerotiniaceae), *Verticillium dahliae* Kleb. (Glomerellales: Plectosphaerellaceae), and *Phytophthora capsici* Leonian (Peronosporales: Peronosporaceae) (Ortiz & Sansinenea, 2023).

Furthermore, *S. marcescens* has been shown to exhibit various characteristics that enhance plant growth and stress tolerance, including phytohormone production, ACC deaminase activity, nitrogen fixation, phosphorus and zinc solubilization, enhancement of antioxidant properties, and modulation of gene expression (Martínez et al., 2018; Soenens & Imperial, 2020; Zheng et al., 2022; Patel et al., 2024). In the present study, this bacterium significantly increased the shoot and root dry weight of potato plants in the absence of pest pressure, corroborating its plant growth-promoting capacity. Despite this beneficial potential, caution is warranted as some *Serratia* species may act as opportunistic phytopathogens (Hasan et al., 2022). The *S. marcescens* (443) utilized in this study was specifically evaluated for potential plant pathogenicity, including the hypersensitive response in tobacco and pectolytic activity in potato, and was determined to be non-pathogenic in these contexts. While these results are encouraging, further studies to comprehensivly rule out any pathogenic potential are recommended prior to field trials. In conclusion, the utilization of this *Serratia* strain appears promissing; however, additional research is required to assess the safety of this and otherspecies concerning human, food, and environmental health, as well as their efficacy against pathogens and pests under *in vivo* conditions.

For microbial biocontrol agents to be successfully integrated into pest and disease management programs, their compatibility with commonly used pesticides is a critical prerequisite. Success depends on the microorganism's ability to maintain its biocontrol efficacy following pesticide application (White et al., 2001). Therefore, a crucial next step is to demonstrate that the endophytic bacteria exhibiting entomopathogenic effects, as established in this study, are not adversely affected by pesticides commonly employed in potato cultivation. Confirming such compatibility would position these bacterial strains as viable components for future integrated pest management strategies.

Conclusion

This study demonstrates that treatments with specific endophytic bacteria can significantly disrupt the feeding of *L. decemlineata* larvae on potato plants The presence of multiple mechanisms of action associated with endophytic bacteria suggests their potential efficacy in biological control strategies targeting plant diseases and pests, as well as in promoting plant growth. Therefore, these endophytic bacteria represent a promissing component for future integrated pest management systems. However, the application of these promising laboratory results to agricultural settings requires validation through field trials. Additionally, comprehensive safety assessments regarding non-target organisms are imperative prior to any practical application.

It is noteworthy that endophytic bacteria, previously recognized for their biocontrol capabilities and plant growth-promoting effects against phytopathogens, exhibited entomopathogenic properties in this study. In our research to date, we have investigated the activities of proteolytic and chitinolytic enzymes, as well as various plant growth-promoting factors, including indole-3-acetic acid (IAA), siderophore production, hydrogen cyanide production, and ACC deaminase activity. However, the mechanisms underlying the entomopathogenic effects of these bacteria on insect hosts, such as toxin and crystal production, warrant further investigation.

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