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Synergistic effects of endophytic bacteria and silicon on controlling common bacterial blight disease in beans

Fasulye bakteriyel adi yanıklık hastalığının kontrolünde endofitik bakteri ve silisyumun sinerjistik etkileri

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A B S T R A C T

Enhancing the effectiveness of environmentally friendly and sustainable practices in plant disease management is crucial for promoting their wider adoption and use. In this context, the combined use of bacterial biocontrol agents and silicon applications holds significant potential. This study aimed to evaluate the effects of individual and combined applications of endophytic bacteria (EB) and silicon on controlling common leaf blight disease caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) in beans. Additionally, the effects of these treatments on plant biomass and chlorophyll content were investigated. Bean plants (*Phaseolus vulgaris* cv. Gina) were grown in a peat/perlite medium under soilless conditions in a climate chamber. Silicon dioxide (SiO_2) (30 mM) and endophytic bacteria were applied to the root collar using the drenching method. The pathogen Xap was inoculated by spraying the leaves, and disease severity was assessed using a 1–5 scale. Plant growth parameters were also recorded. Among the tested EB isolates, *Pseudomonas caspiana* V30G2 was the most effective in suppressing disease severity. Disease severity was reduced by 31% with V30G2 and by 21% with SiO_2 when applied individually. Notably, the combined application of both agents exhibited a synergistic effect, reducing disease severity by 55%. Although some improvements were observed in specific parameters, such as leaf number, neither the individual nor the combined treatments significantly influenced overall plant biomass or chlorophyll content. Nevertheless, the results suggest that the combined application of silicon and endophytic bacteria, when appropriately selected, has significant potential for environmentally friendly and sustainable disease management, enhancing the disease suppression efficacy of each treatment.

INTRODUCTION

In today's intensive agricultural production systems, disease control is essential; however, traditional methods have significant risks to both the environment and human health. While biological control presents promising results in sustainable agricultural practices, its practical application faces several challenges. The inability of biocontrol agents to consistently replicate their laboratory success under field conditions, their susceptibility to environmental fluctuations, and the instability of their activity are critical issues that need to be addressed. In this context, enhancing the effectiveness of biocontrol agents is as crucial as their development.

Plant growth-promoting bacteria (PGPBs) represent one of the most widely studied and applied eco-friendly strategies for plant disease control, though their field efficacy remains limited and requires enhancement. In this regard, silicon has significant potential, both as a direct treatment and in combination with other application methods, to enhance the stability and efficiency of biocontrol agents (Guerriero et al. 2016, Guével et al. 2007, Sahebi et al. 2015).

PGPBs can colonize plants as epiphytes, residing on the plant surface, or as endophytes, inhabiting internal plant tissues. Endophytic bacteria (EB) are defined as microorganisms that can spread throughout the plant without causing harm and spend at least part of their life cycle within plant tissues (Hallmann 1997, Hardoim et al. 2008). EB can promote plant growth and development through both direct and indirect mechanisms (Hardoim et al. 2008, İmriz et al. 2014).

EB contribute directly to plant growth by facilitating nitrogen fixation, enhancing phosphorus solubilization, promoting the uptake of iron and other nutrients, and producing phytohormones (Grobelak et al. 2015). Additionally, they play an indirect role in plant protection by activating mechanisms such as antibiosis, competition, hyperparasitism, and plant-induced resistance or tolerance against harmful organisms (Santoyo 2016).

Compared to epiphytic bacteria, endophytic bacteria offer several advantages. Because they reside within plant tissues, their metabolites can interact directly with and be readily absorbed by the plant (Akköprü et al. 2021, Romano et al. 2020). Furthermore, endophytic bacteria can reach and influence all plant tissues through the plant's vascular system, thereby exerting more widespread effects (Hardoim et al. 2008, Mercado-Blanco and Lugtenberg 2014, Romano et al. 2020).

Silicon (Si) is second only to oxygen in abundance in the Earth's crust (Kim et al. 2002). Vermiculite, smectite, kaolin, orthoclase, feldspars, plagioclase (silicates in the form of crystal), amorphous silica, and quartz are the main Si components in most soils structures (Luyckx et al. 2017, Sahebi et al. 2015). The major soluble forms of Si in the soil are poly- and monosilicic acids. It converts silicon into forms that the plant can use by decomposing silicate minerals (Sahebi et al. 2015). Some plants such as rice accumulate silicon at rates above 1–5% and are considered as accumulator plants, while others such as tomato, cucumber, maize, barley and soybean accumulate at rates lower than 1% (Sahebi et al. 2015).

Though Si addition via silicate slags or solutions to soils or nutrient solutions is common, interest in foliar applications remains high due to their ease of use (Guével et al. 2007). Studies indicated that many plant disease caused by bacteria and fungi, are less severe when silicon is made available resulting in slower disease progress and less disease severity (Fortunato et al. 2015, Guerriero et al. 2016, Luyckx et al. 2017). Rodrigues et al. (2015) indicated that although foliar-applied Si is effective in reducing some foliar diseases, applying silicon to the roots is more effective. Many studies conducted in recent years have shown that silicon has direct or indirect positive effects on the development and functions of plants (Guerriero et al. 2016, Luyckx et al. 2017, Savant et al. 1997). This shows that Si can be included in environmentally friendly sustainable practices in disease control.

In this context, bean, which we have chosen as a model plant, is an important crop and we can test our hypothesis by using EB and Si together in controlling bacterial blight disease, which causes great losses.

Bean (*Phaseolus vulgaris*) is one of the most produced legumes due to their high nutritional content and their significant contributions to soil (Duman and Soylu2019). Türkiye ranks in the top ten producers of beans producers (FAO 2023). The beans are negatively impacted by many bacterial, fungal, and viral pathogens. One of the most important bean bacterial diseases in worldwide and Türkiye that causes significant economic losses is the "common leaf blight" caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) (Bozkurt 2009, Karavina et al. 2011). The presence of this pathogen has been documented in various studies conducted in countries with commercial bean production (Osdaghi 2014).

The Xap is prevalent in temperate and tropical climates, leading to substantial yield losses in bean cultivation regions (Gilbertson and Maxwell 1992, Saettler 1989). The bacteria enter the plant through natural openings and wounds (Ertekin et al. 2016). While the pathogen triggers infections in all above-ground parts of the plant, the symptoms are more severe in leaves and pods. Leaf symptoms appearance of a typical narrow, lemon-yellow halo around it (Rudolph 1993, Vidaver 1993). Beyond leaf symptoms, the pathogen also causes issues in fruits, reducing seed germination in severe cases (Ertekin et al. 2016)

For disease control, cultural measures are typically implemented. Additionally, chemical applications like foliar and seed spraying with copper compounds are recommended (Bozkurt 2009, Schwartz et al. 2007). However, chemical spraying alone is inadequate in preventing fruit infections and may not yield satisfactory results (Opio et al. 1996, Park et al. 1999). The use of antibiotics has been restricted in several countries, and the pathogen's rapid development of resistance to antibiotics and copper preparations has been observed (İmriz et al. 2014).

In this context, within the framework of an environmentally friendly and sustainable agricultural approach, the use of appropriate combinations can enhance the effectiveness of biological control microorganisms or applications holds significant potential. This study aimed to evaluate the potential of single or combined applications of endophytic bacteria (EB) and silicon (Si) in controlling "common leaf blight" disease caused by *Xanthomonas axonopodis* pv. phaseoli in beans.

MATERIALS AND METHODS

Plant material and plant growth medium

Bean plants (*Phaseolus vulgaris* cv. Gina) were cultivated under soilless conditions using a peat/perlite (1:1, v/v) substrate. Seeds were sown into 250 ml plastic pots filled with the growth medium and maintained in a climate-controlled chamber set at 25 ± 2 °C, with a photoperiod of 14 hours and approximately 50% relative humidity (Figure 1a). To ensure adequate nutrition, a nutrient solution formulated according to the composition outlined in Table 1 was supplied regularly throughout the experiment (Hoagland and Arnon 1950). The study was carried out in the climate chambers of Van Yüzüncü Yıl University, Faculty of Agriculture, Plant Protection Department in 2020.

Endophytic bacteria and its application

Endophytic bacteria (EB) were selected from isolates whose some properties were determined by previous studies (Babier and Akköprü 2020, Olur 2019) (Table 2). *Pseudomonas fluorescens WCS365* isolate, have some PGP traits and it is trigger plant immunity (Bolwerk et al. 2003, Kamilova et al. 2005), was used as reference isolate obtained from Dr. Kamilova. EB were cultivated in King's-B medium (KB) (peptone 20 g/l, K_2HPO_4 1.5 g/l, $MgSO_47H_2O$ 1.5 g/l, glycerol 10 ml/l, agar 15 g/l) and incubated at 27 °C for 24 hours. Bacterial suspensions were prepared from the colonies, adjusted to an OD_{600} of 0.1 (approximately 10^8

Figure 1. Bean seedlings prepared for applications (a), application of SiO_2 and Endophytic bacteria to the root collar by drenching method (b), patogen *Xanthomonas axonopodis* pv. *phaseoli* application to the seedlings by pulverization (c), *in vitro* antagonistic test (d).

Table 1. Composition of nutrient solution used to meet the nutritional needs of beans seedlings according to recipt of Hoagland and Arnon (1950)

A nutrient solution (%)		**B nutrient solution (%)***	
Total Nitrogen (N)	10.3	Total Nitrogen (N)	2.1
Ammonium (NH4)	1.6	Nitrate (NO3)	2.1
Nitrate (NO_3)	8.7	Potassium oxide (K2O)	11.6
Potassium oxide (K_2O)	7.5	Phosphorus pentoxide (P2O5)	6.4
Calcium (Ca)	8.6	Magnesium (Mg)	1.6
Iron DTPA (Fe)	0.3	Manganese (Mn)	0.1
		Zinc (Zn)	0.01
		Boron (B)	0.03
		Copper (Cu)	0.003
		Molybdenum (Mo)	0.004

CFU/ml) using a spectrophotometer. The prepared the EB suspensions were applied to each seedling by drenching from the root collar of the plants as 20 ml (Figure 1b). EB application was done twice; the first application was carried out 8 days after planting, and the second application was carried out 10 days later. The groups that did not receive EB application were given water in the same amount and method.

Determination of disease severity

Disease symptoms observed 21 days after the inoculation with the pathogen Xap were evaluated using a 1–5 disease severity scale. The scale was defined as follows: 1 = no symptoms; 2 = necrosis or isolated spots affecting 1–5% of the leaf area; 3 = symptoms and necrosis affecting 6–25% of the leaf; 4 = symptoms and necrosis affecting 26–50% of the leaf; and 5 = symptoms, necrosis, or complete leaf death affecting more than 50% of the leaf surface (Akköprü 2020). The recorded scale values were converted into disease severity percentages using the Townsend Heuberger formula (1) (Townsend and Heuberger 1943). The percentage of disease reduction was calculated as the relative difference in disease severity between the treatment and the control groups.

$$DS(\%) = \sum ((S \times L) / (M \times Smax)) \times 100. \qquad (1)$$

where in DS: disease severity, S = scale value, L = the number of plant leaves evaluated in each scale, M = the total number of plant leaves, and Smax = the highest scale value.

Table 2. The endophytic bacteria, (EB) and their plant growth promoting traits, isolated and identification by Kamilova et al. (2005), Olur (2019), Babier and Akköprü (2020)

EB isolates	NCBI Acs. Num.	ACC-d	Sid (mm)	P (mm)	IAA (ppm)
Pseudomonas caspiana V30G2	(MN128080)	-	4.50	+	-
Pantoea sp. V31Y4	(MT249279)	3	2.25	-	25.03
Pseudomonas fluorescens WCS365	-	ND	6	+	8
Bacillus velezensis V40K2	MN186863	3	7.00	-	1.38
(ND) G116S2	-	-	1.25	+	ND

*EB: Endophytic bacteria, ACC-d: 1-aminocyclopropane-1-carboxylate deaminase, Sid: Siderophore activity, P: Phosphate solubilizing activity, IAA: Indole-3-acetic acid, NCBI Acs. Num.: NCBI Genbank Accession number, ND: Undetermined

Silicon and its application

The Silicon dioxide (SiO_2) (Si) form of silicon was used in the study. Si was applied to the plant once using the drenching method (Çelik 2021, Çelik and Akköprü 2025). Si suspensions were prepared at 30 mM concentrations with the help of sterile pure water. The 15 ml of Si suspensions per plant was applied into the root collar by the drenching method (Figure 1b). Si was applied 9 days after seed sowing, when the dual leaves were fully open and the triple leaves were forming.

Pathogen and its application

In the study, *Xanthomonas axonopodis* pv. *phaseoli* (Xap), whose virulence was determined and used in previous studies, was used (Akköprü 2020). A 48-hour Xap culture grown in KB medium was adjusted to 10^8 CFU/ml using a spectrophotometer, with 0.01% Tween added. The suspension was uniformly applied to leaves via hand sprayer 48 hours post-Si and 24 hours post-EB application (Figure 1c). Only sterile water was sprayed on the leaves of plants that did not receive pathogen application. After the application, the plants were covered with a transparent polyethylene cover for 48 hours and exposed to high relative humidity and kept in a lightless environment for the first 24 hours. Then, the plants were left to develop in the climate chamber under normal conditions (with a photoperiod of 14 hours and approximately 50% relative humidity and at 25±2 °C).

Determination of plant growth parameters and total chlorophyll content

Leaf number was recorded on day 21 at the end of the experiment. Bean plants were uprooted and cleaned of the growing medium residues by washing. The shoot fresh weights were determined by cutting them from the root collar and weighing them. After the roots were dried with the help of blotting papers, they were weighed and their fresh weights were determined. The plant roots and shoots, whose fresh weights were determined, were placed in aluminum foil containers and dried in an oven at 65 °C for 48 hours, and then weighed and their dry weights were determined.

A chlorophyll meter (Minolta brand SPAD) device was used to determine the total chlorophyll content in the leaves of bean seedlings treated with Xap, SiO_2, and EB. For this purpose, readings were taken from three leaves of the same age in each seedling, and the chlorophyll values were determined by taking the average of the three readings.

In vitro antagonistic effect

In this study, the in vitro antagonistic effects of SiO_2 on EB and Xap, as well as the effect of EB on Xap, were investigated. For this purpose, 100 µl of a Xap suspension with a concentration of 10^6 CFU/ml was evenly spread onto King's B (KB) agar medium and allowed to dry. Similarly, 100 µl of an EB suspension, adjusted to an optical density (OD) of 0.05 using a spectrophotometer, was spread on a separate KB medium plate and dried. Following the drying process, four sterile paper disks were placed equidistantly on the surface of each prepared Petri dish. Subsequently, 10 µl of either SiO_2 or EB suspension was applied to each disk. After a two-day incubation period, inhibition zones or Xap growth around the disks were examined (Figure 1d).

Data analysis

Experiments were repeated twice. The obtained data were subjected to variance analysis with the help of SPSS (SPSS, Inc. 2007) software package, and the means were evaluated with Duncan's multiple comparison test. Each treatment group had three replications, and at least 15 seedlings were used in each replication. The study was conducted according to Table 3.

Table 3. Treatment groups designed according to randomized plots experimental design

1) NC	5) PC
2) EB	6) EB+*Xap*
3) SiO_2	7) SiO_2+*Xap*
4) EB+SiO_2	8) EB+SiO_2+*Xap*

*SiO_2: Silicon dioxide, EB: Endophytic bacteria, Xap: *Xanthomonas axonopodis* pv. *phaseoli*, PC: positive control, NC: Negative Control

RESULTS

In vitro antagonistic effect of Si and EB

Certain EB isolates exhibited antagonistic activity against *Xanthomonas axonopodis* pv. *phaseoli* (Xap). Among these, isolates V40K2 and V30G2 demonstrated the highest levels of inhibition, with inhibition zone diameters of 13.5 mm and 10 mm, respectively (Figure 1d). In contrast, silicon (Si) at a concentration of 30 mM showed no observable antagonistic effect on either Xap or the EB isolates.

EB selection

The most successful EB isolate was selected according to its level of disease suppression and contribution to shoot development. It was observed that all EB isolates used in the studies suppressed the disease caused by Xap between 38 and 51% (Figure 2). Among these isolates, V30G2 with disease severity values of 10.87 and G116S2 with 10. respectively, V30G2 and G116S2 stood out (Figure 2).

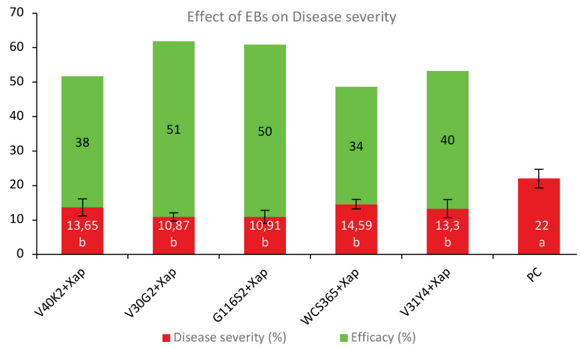

Figure 2. Effect of endophytic bacteria (EB) on disease severity (red parts of columns) of common leaf blight disease caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) on bean seedlings and efficacy percentage (green parts of columns) of EB treatment on disease severity

* Values with the same letter in the column are not signifcantly different when followed by the Duncan's multiple range test at $P < 0.05$

On the other hand, none of the isolates used made significant contributions to the number of leaves (LN), shoot fresh (SFW) and dry (SDW) weight (Table 4). In fact, it was observed that the G116S2 isolate reduced LN and SFW. Similar effects were observed under disease pressure (Figure 2).

In light of these data, EB V30G2 isolate was selected to be used in the next stages of the study. This selected isolate was used with 30 mM SiO2 to investigate its potential to control the disease.

Effect of SiO_2 and EB V30G2 isolate on disease severity and plant development

In this stage of the study, the effects of SiO_2 and EB V30G2 single and combined applications on disease caused by Xap and plant development were investigated. All application groups showed an effect between 21 and 55% in suppressing disease severity. Single applications of SiO_2 and EB V30G2 reduced disease severity. However, the decrease obtained from V30G2+Xap application was found to be statistically significant. In addition, the combined use of SiO_2 and EB V30G2 reduced disease severity by 55%, more than the individual effect of both applications (Figure 3).

Table 4. Effects of endophytic bacteria (EB) application on leaf number, shoot fresh weight and shoot dry weight of bean seedlings treated and untreated with pathogen *Xanthomonas axonopodis* pv. *phaseoli*

Treatments	LN	SFW (g)	SDW (g)
NC	17.87±0.99 a	6.38±0.44 ab	0.69±0.10 a
EB V40K2	14.80±1.54 cde	5.28±0.88 def	0.50±0.10 de
EB V30G2	14.30±1.25 de	5.33±1.15 def	0.50±0.13 de
EB G116S2	14.10±1.66 e	4.96±1.39 ef	0.49±0.11 de
EB WCS365	16.40±1.71 abcd	5.74±0.80 bcdef	0.58±0.05 bcde
EB V31Y4	15.30±2.21 bcde	4.73±1.03 f	0.46±0.13 e
PC	16.00±2.00 bcde	6.69±1.20 a	0.66±0.14 bc
EB V40K2+*Xap*	15.40±3.09 abcde	5.74±0.90 bcdef	0.52±0.10 de
EB V30G2+*Xap*	14.30±2.94 de	5.55±1.34 cdef	0.46±0.16 e
EB G116S2+*Xap*	14.90±1.85 bcde	4.95±0.90 ef	0.46±0.09 e
EB WCS365+*Xap*	16.66±1.11 abc	5.74±0.73 bcdef	0.54±0.09 cde
V31Y4+*Xap*	17.10±3.17 ab	5.99±1.41 bcde	0.55±0.15 cde

*EB: Endophytic bacteria, Xap: *Xanthomonas axonopodis* pv. *phaseoli*, SFW: Shoot fresh weight, SDW: Shoot dry weight, LN: Leaf number, NC: Negative Control, PC: Positive control (only Xap applied). Values with the same letter in each column are not signifcantly different when followed by the Duncan's multiple range test at P < 0.05

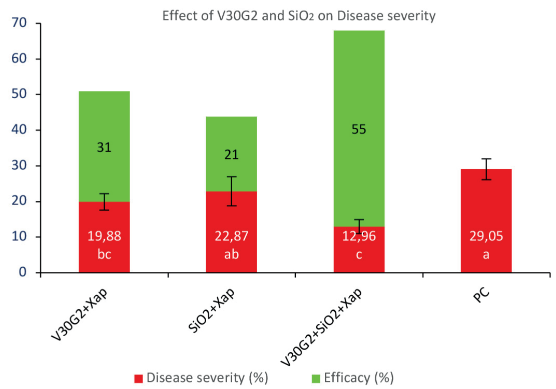

Figure 3. Effect of endophytic bacteria (EB) and silicon (red parts of columns) treatment on severity of common leaf blight disease caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) on bean seedlings and efficacy percentage (green parts of columns) of this treatment on disease severity

* Values with the same letter in each column are not signifcantly different when followed by the Duncan's multiple range test at P < 0.05

SiO_2 and EB V30G2 isolate applied individually and in combination did not show a significant effect on shoot fresh, shoot dry and root dry weight. However, single and combined applications of the agents significantly increased root fresh weight (Table 5).

Under disease pressure, single or combined applications of SiO_2 and EB V30G2 did not show a significant effect on root and shoot fresh/dry weights (Table 5). It was determined that SiO_2 and EB V30G2 isolate applied individually and in combination did not have a significant effect on leaf number and chlorophyll content. However, it was determined that leaf number increased significantly in the group where SiO_2 and EB V30G2 were used together under disease pressure (V30G2+SiO_2+Xap) compared to the group where only pathogen was applied (PC).

In the groups where there was no disease pressure, the applications did not cause an increase in total chlorophyll content. On the other hand, SiO_2 application under disease pressure significantly increased total chlorophyll content compared to PC (Table 6).

DISCUSSION

Biological control agents (BCAs) used in the management of plant diseases may exhibit limitations in their biocontrol efficacy due to inherent biological characteristics. For instance, their performance can be markedly influenced by environmental fluctuations, and their interactions with various host plants may vary, potentially restricting their effectiveness under field conditions. These challenges can undermine the reliability and broader adoption of biocontrol strategies based on BCAs. Therefore, enhancing the efficacy of BCAs remains a critical objective (Spadaro and Gullino 2005). In this regard, supplementing BCA applications with supportive agents may offer promising results. Among these, silicon (SiO_2) has attracted considerable attention due to its potential role in enhancing plant resistance and microbial efficacy. In the present study, the potential of silicon (SiO_2) to enhance the biocontrol effectiveness of endophytic

Table 5. Effects of endophytic bacteria (EB) V30G2 and SiO2 application on leaf number, shoot and root fresh/dry weight of bean seedlings treated and untreated with pathogen *Xanthomonas axonopodis* pv. *phaseoli*

Treatments	SFW (g)	SDW (g)	RFW (g)	RDW (g)
NC	0.68±0.09 a	6.66±0.76 a	0.52±0.30 c	0.08±0.01 a
V30G2	0.55±0.22 ab	5.86±1.56 ab	1.21±0.67 ab	0.06±0.02 ab
SiO_2	0.58±0.16 ab	5.72±1.73 ab	1.62±0.49 a	0.08±0.02 a
V30G2+SiO_2	0.66±0.19 ab	6.49±1.45 a	1.64±0.59 a	0.07±0.04 ab
PC (Xap)	0.51±0.12 b	4.57±0.97 b	1.06±0.15 b	0.05±0.01 b
V30G2+*Xap*	0.55±0.14 ab	4.82±1.25 b	1.11±0.63 b	0.06±0.02 ab
SiO2+*Xap*	0.63±0.15 ab	5.52±1.34 ab	0.97±0.19 b	0.06±0.01 ab
V30G2+SiO_2+*Xap*	0.65±0.10 ab	5.80±1.10 ab	1.18±0.28 b	0.07±0.02 ab

*EB: Endophytic bacteria, SiO_2: 30 mM silicon dioxide, Xap: *Xanthomonas axonopodis* pv. *phaseoli*, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, NC: Negative Control, PC: Positive control (only Xap applied). Values with the same letter in each column are not signifcantly different when followed by the Duncan's multiple range test at $P < 0.05$

Table 6. Effects of endophytic bacteria (EB) V30G2 and SiO_2 application on leaf number and total chlorophyll content of bean seedlings treated and untreated with pathogen *Xanthomonas axonopodis* pv. *phaseoli*

Gruplar	LN	Chlorophyll
NK	18.80±2.09 ab	39.29±3.66 a
EB V30G2	19.30±4.02 ab	36.85±6.12 ab
SiO_2	17.40±3.33 ab	37.36±4.10 ab
EB V30G2+SiO_2	17.40±4.57 ab	39.21±5.51 a
PK	16.00±2.74 b	34.60±3.06 b
EB V30G2+*Xap*	18.44±2.87 ab	36.36±2.23 ab
SiO2+Xap	19.11±3.25 ab	40.53±3.27 a
EB V30G2+SiO_2+*Xap*	19.90±1.52 a	37.98±2.19 ab

*EB: Endophytic bacteria, Xap: *Xanthomonas axonopodis* pv. *phaseoli*, LN: Leaf number, Chlo: total chlorophyll content, NC: Negative Control, PC: Positive control (only Xap applied). Values with the same letter in each column are not signifcantly different when followed by the Duncan's multiple range test at $P < 0.05$

bacteria against common bacterial blight disease, caused by *Xanthomonas axonopodis* pv. *phaseoli* in bean plants, was investigated.

Many studies have been conducted to control common leaf blight disease in beans with the help of PGPRs or EB and successful results have been obtained (Belete et al. 2021, Corrêa et al. 2017, Sallam and Aldayel 2025). Bozkurt (2009) showed that antagonistic bacteria isolated from bean plants inhibited the disease by 42-72% in capsule tests, 32-67% in pot tests and 30-55% in field tests. It was observed that the five EB isolates we used in our study suppressed the disease severity between 34 and 51% (Figure 2). Among these isolates, *Pseudomonas caspiana* V30G2, the most successful isolate in disease suppression, was selected to be used together with Si. The disease suppression ability of the V30G2 isolate may be due to its antagonistic effect. This is because *in vitro* tests have determined that the V30G2 isolate has an antagonistic effect against Xap (Figure 1d). However, it may have used other biocontrol mechanisms and/or triggered induced plant resistance. There are many studies on the abilities of *Pseudomonas* sp. in this direction (Alattas et al. 2024).

Silicon protects the plant against biotic stresses such as plant diseases and insect pests as well as abiotic stress and helps its development (Ma 2004, Sahebi et al. 2015, Sistani et al. 1997, Rajput et al. 2021). Although the effectiveness of silicon varies depending on its form, application method and dose, there are many publications indicating that it suppresses many plant diseases (Luyckx et al. 2017, Sahebi et al. 2015). Previous studies have reported that silicon application significantly suppresses diseases caused by *Meloidogyne incognita*, *Pectobacterium betavasculorum*, *Rhizoctonia solani*, *Xanthomonas axonopodis* pv. *phaseoli*, and *Pseudomonas syringae* pv. *tomato* (Andrade et al. 2013, Çelik and Akköprü 2025, Khan et al. 2020, Shetty et al. 2011, Siddiqui et al. 2020). Consistent with these findings, our study also demonstrated a 34% reduction in disease severity following silicon treatment.

Si has been reported to accumulate in plant cell walls, where it contributes to stress tolerance by forming a physical barrier against various biotic and abiotic stress factors (Luyckx et al. 2017, Sahebi et al. 2015). Additionally, Si enhances plant defense mechanisms by stimulating the activity of defense-related enzymes such as peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, and lipoxygenase, thereby restricting pathogen penetration (Cai et al. 2008, Chérif et al. 1994, Fauteux et al. 2005; Polanco et al. 2012, Shetty et al. 2011). Furthermore, Si is also reported to modulate the content and activity of plant hormones, influencing physiological responses to stress (Luyckx et al. 2017).

Despite these well-documented roles of Si in enhancing plant resistance, our study did not reveal a significant effect of Si on plant growth parameters. Similarly, previous studies have reported that Si applications can increase resistance to biotic stress without necessarily promoting overall plant development (Çelik and Akköprü 2025, Guével et al. 2007).

The aim of combining various control methods in control plant diseases is to obtain a synergistic effect rather than just an additive. In this context, the use of Si, which is safe for human health and the environment, together with BCAs has great potential (Etesami 2024, Sahebi et al. 2015). One of the important factors here is to be able to create appropriate combinations.

Some studies have shown that the combined use of PGPR and Si can protect the plant under abiotic stress and support its development. Mahmood et al. (2016) the combined application of the *Bacillus drentensis* with Si resulted in the greatest enhancement of mung bean physiology, growth, and yield under the salinity stress (Mahmood et al. 2016). Also, Kubi et al. (2021) reported that the application of *Pseudomonas psychrotolerans* CS51 + Si combinations was the most successful application by significantly increasing maize biomass and chlorophyll content under salinity stress.

However, neither our literature review nor that conducted review by Etesami (2024) revealed any studies investigating the combined use of silicon (Si) and biological control agents (BCAs) against plant aboveground diseases. The findings demonstrated that the combined application of the endophytic bacterial isolate V30G2 and silicon dioxide (SiO_2) shown the synergistic effect and significantly enhanced disease suppression compared to individual treatments. While individual applications of the EB V30G2 and SiO_2 resulted in 31% and 21% disease suppression, respectively, their combined application achieved a suppression rate of 55% (Figure 3).

This synergistic effect may have resulted from the activation of plant-induced resistance mechanisms. PGPR have long been recognized for their capacity to induce plant resistance. In recent decades, however, growing evidence has demonstrated that Si also plays a crucial role in enhancing plant defense responses. Silicon contributes to plant resistance against pathogenic infections by upregulating defense-related gene expression; increasing the synthesis of phenolic compounds, callose, phytoalexins, and lignin; and boosting levels of polyphenols, antimicrobial flavonoids, and anthocyanins (Verma et al. 2022). Notably, Kubi et al. (2021) reported that the combined application of the biological control agent *Pseudomonas psychrotolerans* CS51 and silicon significantly reduced abscisic acid (ABA) levels by 1.5-fold and jasmonic acid (JA) content by 14.89%. Furthermore, this co-application enhanced the antioxidant system by increasing flavonoid content by 97% and polyphenol content by 19.64%.

On the other hand, microbial biocontrol agents can facilitate the uptake of Si, which is difficult to absorb and has low solubility in soil. Bacteria belonging to genera such as *Enterobacter*, *Pseudomonas*, *Proteus*, *Bacillus*, *Rhizobia* and *Burkholderia* are known to increase the uptake of Si in soils (Etesami and Jeong 2022, Raturi et al. 2021). This may contribute to plant protection. Sahebi et al. (2015) stated that increasing the Si content in the plant body was related to the decrease in disease occurrence.

The *Pseudomonas caspianhalide* V30G2 isolate used in our study has the ability to solubilize phosphate (Table 2) (Babier and Akköprü 2020). Also, it is known that phosphate-solubilizing bacteria affected the availability of Si and increase the uptake of this element by plants (Etesami et al. 2021). Different bacterial species are known to degrade various types of silicates using mechanisms such as acid production, exopolysaccharide secretion, and chelate-forming metabolites (Etesami 2024, Rezakhani et al. 2022). Phosphate-solubilizing bacteria (PSB) facilitate P uptake by plants through organic P mineralization and solubilization of insoluble mineral phosphates (Sharma et al. 2013). PSBs do this by reducing soil pH and producing organic acids and phosphatases. Similarly, silicon-solubilizing bacteria promote silicate dissolution by creating acidic conditions and producing various organic acids involved in this process (Etesami 2024, Etesami et al. 2021). Rezakhani et al. (2022) observed that the application of silicon (Si) in combination with PSB isolates, such as *Pseudomonas* sp. FA1 and *Bacillus simplex* UT1, enhanced the availability of Si forms that can be absorbed by plants. Furthermore, the study revealed that the combined application of Si and PSB strains significantly increased Si accumulation in plant shoots compared to both the control group and the individual applications of Si or PSB.

In addition, as is known, the rhizosphere is one of the most effective elements in maintaining soil properties and plant health. In this context, silicate-solubilizing bacteria in the soil can convert insoluble silicates into soluble Si (Rajput et al. 2021). This can contribute to the plant by changing the soil microbiota. In fact, it has been determined that Si applications can increase microbial biomass in soils and plants' access to Si (Rajput et al. 2021).

The combined application of EB and Si did not cause a significant increase in other growth parameters except for the number of leaves. Guével et al. (2007) and Çelik and Akköprü (2025) also reached similar findings.

In conclusion, it is well-documented that Si and plant PGPRs individually enhance plant resistance to various biotic and abiotic stress factors or mitigate their adverse effects (Etesami 2024, Rajput et al. 2021). However, studies investigating the combined application of Si and PGPRs against biotic diseases remain scarce or non-existent. In this context, the findings of the present study are considered original. Our results demonstrate that the combined application of Si and EB provides greater protection against common bacterial blight of bean caused by *Xanthomonas axonopodis* pv. *phaseoli* compared to their individual use. Nonetheless, this combined treatment did not yield a significant improvement in overall plant growth. These findings suggest that the strategic combination of an appropriate Si dose and form with effective EB or PGPR isolates holds considerable promise for environmentally friendly and sustainable agricultural practices.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The author declared no conflict of intere

ÖZET

Bitki hastalıklarının yönetiminde çevre dostu ve sürdürülebilir uygulamaların etkinliğini artırmak, onların daha geniş çapta benimsenmesi ve kullanımını teşvik etmek için çok önemlidir. Bu bağlamda, bakteriyel biyokontrol ajanları ile silisyumun (Si) birlikte kullanılması önemli bir potansiyel taşımaktadır. Bu çalışma, fasulyelerde *Xanthomonas axonopodis* pv. *phaseoli* (Xap)'nin neden oluğu adi yaprak yanıklığı hastalığının kontrolünde Endofitik Bakteriler (EB) ve silisyumun teksel ve birlikte uygulamalarının etkilerini belirlemeyi amaçlamıştır. Ek olarak, bu uygulamaların bitki biyokütlesi ve klorofil içeriği üzerindeki etkileri araştırılmıştır. Fasulye (*Phaseolus vulgaris* cv. Gina) fideleri, iklim odasında topraksız tarım sisteminde torf ve perlitten oluşan yetiştirme ortamında geliştirilmiştir. Silisyum dioksit (SiO_2) (30 mM) ve EB, içirme yöntemi kullanılarak kök boğazına uygulanmıştır. Patojen Xap, yapraklara püskürtülerek uygulanmış ve hastalık şiddeti 1-5 skalası kullanılarak değerlendirilmiştir. Test edilen EB arasında, *Pseudomonas caspiana* V30G2 hastalık şiddetini baskılamada en etkili izolat olmuştur. Teksel uygulamalarda hastalığın şiddeti V30G2 ile %31, SiO_2 ile %21 düzeyinde azaltılmıştır. Ancak, her iki etkenin birlikte uygulanması sinerjistik bir etki göstererek hastalık şiddetini %55 oranında azaltmıştır. Yaprak sayısı gibi belirli parametrelerde bazı pozitif etkiler gözlemlenmiş fakat ne tek başına ne de birlikte yapılan uygulamalar genel bitki biyokütlesini veya klorofil içeriğini önemli ölçüde etkilememiştir. Sonuç olarak, uygun şekilde seçilmiş silikon ve endofitik bakterilerin birlikte uygulanmasının çevre dostu ve sürdürülebilir hastalık yönetimi için önemli bir potansiyele sahip olduğunu ve her bir uygulamanın hastalık baskılama etkinliğini artırdığını göstermektedir.

Anahtar kelimeler: biyokontrol, *Xanthomonas axonopodis* pv. *phaseoli*, *Pseudomonas caspiana*, silisyum dioksit

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