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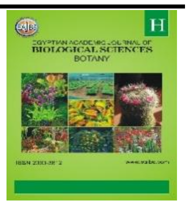

Evaluation of *Bacillus subtilis* As a Biocontrol Agent Against Viral Pathogens in Faba Bean (*Vicia faba*)

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ABSTRACT

Two field experiments were carried out at The Faculty of Agriculture (Saba Basha), Alexandria University, Egypt during 2023/2024 and 2024/2025 seasons to assess the effectiveness of *Bacillus subtilis* as a biological control agent in reducing the incidence and severity of viral infections (BYMV) in faba bean and its effects on vegetative growth. The experiment was conducted in a greenhouse over two consecutive seasons using a randomized complete block design (RCBD) in three replicates, with foliar application of *B. subtilis* at concentrations of 0.5, 1.0, 1.5, and 2.0 g/L, along with a control significantly improved the vegetative growth and yield of faba bean, especially when applied before viral infection. The 1.5 g/L pre-infection treatment resulted in the highest level of effectiveness among all treatments. The results indicated that foliar application of *B. subtilis* extract increased values in plant height, number of pods, and pod weight during both seasons, suggesting strong preventive effects. In contrast, virus-infected plants without treatment had the lowest performance across all traits. Treatments applied for post- infection gave moderate improvements but were less effective than preventive applications. These findings highlight the role of *B. subtilis* as a biocontrol agent that enhances plant immunity and growth under stress. It is recommended to apply *B. subtilis* preventively at 1.5 g/L for optimal protection and productivity in faba bean cultivation. ELISA is a sensitive and reliable technique used to detect viral infections in plants by measuring viral protein concentrations. It helps evaluate the effectiveness of biological treatments in reducing virus presence.

INTRODUCTION

Legume crops play a crucial role in global agriculture due to their ability to fix atmospheric nitrogen, improve soil fertility, and provide high-protein food for both humans and animals. However, the productivity of leguminous plants is frequently threatened by a variety of pathogens, particularly plant viruses. Faba bean (*Vicia faba* L.) is a vital leguminous crop cultivated for its high protein content and soil-enriching nitrogen-fixation properties.

Faba bean is one of the oldest cultivated legume crops and is extensively grown in Mediterranean, African, and Middle Eastern countries. Its importance as a food legume lies in its adaptability, soil enrichment through biological nitrogen fixation, and contribution to

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food security in developing regions (Duc *et al*., 2010). Despite its agronomic and nutritional benefits, faba bean production faces serious challenges, particularly from viral pathogens.

Viral diseases can cause substantial yield losses, delayed development, and reduced seed quality, ultimately affecting the economic value of the crop (Hull, 2014). Common viruses infecting legumes include Bean common mosaic virus (BCMV), Pea seed-borne mosaic virus (PSbMV), and Alfalfa mosaic virus (AMV), all of which are difficult to control due to the lack of direct antiviral agents for plants and the wide range of vectors, such as aphids, that transmit them (Brunt *et al*., 1995). Major viruses infecting faba beans include Bean yellow mosaic virus (BYMV), Faba bean necrotic yellows virus (FBNYV), Pea seed-borne mosaic virus (PSbMV), Broad bean stain virus (BBSV), and Broad bean mottle virus (BBMV) (Horn *et al*., 2011). Bean yellow mosaic virus (BYMV) is the most dangerous plant virus, causing massive crop yield quality and productivity losses. BYMV lowers the global faba bean yield and restricts photosynthetic activity (Miteva *et al*., 2005). BYMV infections in faba bean plants reduced yield production by 17–59% (Omar 2021). These viruses can cause systemic symptoms such as chlorosis, necrosis, mottling, stunting, and deformation of leaves and pods. Yield losses due to viral infections can exceed 50%, especially under conditions that favor vector proliferation (e.g., aphids, whiteflies) (Makkouk *et al*., 2012).

As chemical control options are limited and undesirable due to environmental concerns, biological control using beneficial microbes like *B. subtilis* has emerged as a promising alternative. In response to these challenges, researchers have increasingly turned to environmentally sustainable strategies, including biological control, to manage plant pathogens. One promising avenue is the use of plant growth-promoting rhizobacteria (PGPR), particularly *B. subtilis*, a gram-positive, spore-forming bacterium known for its resilience in diverse environments and its ability to form beneficial associations with plant roots (Kloepper *et al*., 2004). This bacterium produces a wide range of bioactive compounds, such as lipopeptides and antibiotics, and has been shown to induce systemic resistance in plants, which enhances the plant's ability to withstand various pathogens, including fungi, bacteria, and viruses (Chen *et al.*, 2007).

Bacillus subtilis may not only act as a growth enhancer but also possess antiviral properties. For example, *B. subtilis* has been reported to activate plant defense signaling pathways, such as the salicylic acid (SA) and jasmonic acid (JA) pathways, which are involved in systemic acquired resistance (SAR) and induced systemic resistance (ISR), respectively (Kumar *et al*., 2012). These pathways are crucial in limiting viral replication and movement within the plant tissues. Furthermore, the production of specific enzymes such as chitinases and glucanases by *B. subtilis* contributes to the degradation of viral vectors and inhibition of viral particles (Meena *et al*., 2017). The use of *B. subtilis* in viral disease management on legume crops remains underexplored. The variability of plant-microbe-virus interactions, environmental factors influencing bacterial colonization, and the specificity of viral inhibition mechanisms require further investigation. Understanding how *B. subtilis* interacts with leguminous plants and their viral pathogens is essential for optimizing its application as a biocontrol agent in the field. The bacterium is known to produce volatile organic compounds (VOCs) that act as signaling molecules, stimulating the plant’s immune system and enhancing resistance to biotic stresses, including viral infections (Sharma *et al*., 2014). Furthermore, *B. subtilis* can produce lipopeptides and polyketides, which have been shown to inhibit viral replication directly or indirectly through enhancing the plant's defense responses (Vidal *et al*., 2016). Several studies have explored different application concentrations to determine the optimal dose for antiviral effects. For instance, a study by Guo *et al*. (2017) evaluated the efficacy of various concentrations of *B. subtilis* for controlling virus-induced diseases in legume plants. The study found that lower concentrations (e.g., 0.5 g/L) were effective in reducing viral symptoms, while higher concentrations (e.g., 2 g/L) provided even greater reductions in viral titers but with diminishing returns.

In this respect**,** Yu and Yu (2019) discussed the possible mechanisms used to suppress a viral attack as well as the use of loclendophytic bacteria for antiviral control in crops. The review describes modern approaches to plant protection against viruses via genome editing, regulation of the expression of the host plant and /or endophytic microorganisms that combine protective activity and immunomodulating potential (Maksimov, *et al*., 2011). The efficacy of *B. subtilis* subsp. *subtilis* was assessed for protection of cucumber and Arabidopsis against Cucumber mosaic virus (CMV). Moreover, transcriptomic analysis was carried out for *B. subtilis* subsp. *subtilis* and infected with CMV (Elsharkawy *et al*., 2021). Furthermore, the increase in the number of pods and pod weight in treated plants reflects the role of *B. subtilis* in promoting flowering and pod formation, possibly through the induction of systemic resistance (ISR) and stimulation of plant metabolism (Kloepper *et al.,* 2004). Even post-infection applications showed improvements compared to untreated infected controls, though the effect was less pronounced than in pre-infection treatments. This supports the hypothesis that *B. subtilis* not only protects plants through early defense activation but also mitigates damage post infection by reinforcing plant immune responses and promoting recovery (Rabileh 2023).

Therefore, the objective of this study is to evaluate the effectiveness of *B. subtilis* as a biological control agent against viral pathogens infecting faba bean (*Vicia faba*) plants.

MATERIALS AND METHODS

Setup of the Experiments*:*

Two field experiments were carried out at the Faculty of Agriculture (Saba Basha), Alexandria University, Egypt during 2023/2024 and 2024/2025 seasons to evaluate the effectiveness of *B. subtilis* as a biological control agent in reducing the incidence and severity of viral infections in growth of faba bean (*Vicia faba* L.) cv Giza 716.

Soil Composition Used in the Experiment:

The soil mixture prepared for the pot consisted of clay and sand in a ratio of 2:1 to provide suitable texture and drainage for optimal growth of faba bean plants. The soil was thoroughly mixed to ensure homogeneity before filling the pots. Some soil properties were determined according to the method described by Page *et al.* (1982) and are presented in Table (1).

***Bacillus subtilis* Extract Analysis:**

The *B. subtilis* extract was analyzed by first culturing the bacteria and preparing a liquid suspension. The viable cell count was determined using serial dilution and plating on nutrient agar. The pH and viscosity of the extract were measured using a pH meter and viscometer, respectively. Protein concentration was assessed using the Bradford assay. Antiviral activity was evaluated through in vitro assays measuring the inhibition percentage of virus growth on plant tissue cultures. Enzyme activity was determined using standard enzymatic assays specific to enzymes of interest (Choudhary and Johri 2009). The activation of *Bacillus subtilis* shown in Figure 1.

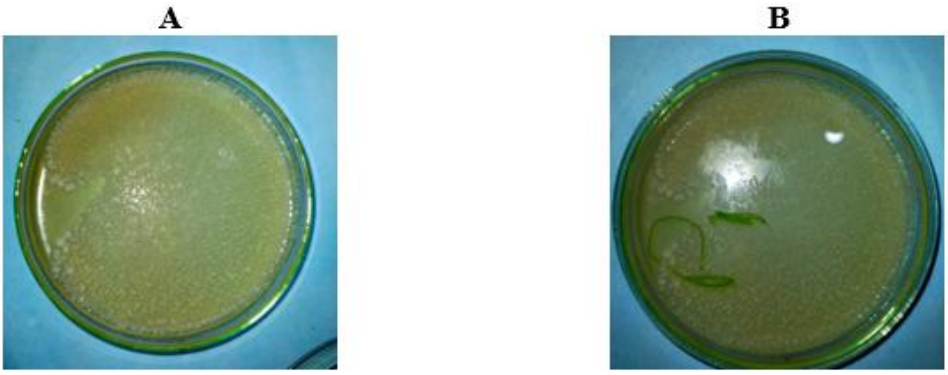

Fig. 1. A and B show the *Bacillus subtilis activity test*

Table 1. Soil Physical and chemical properties of experimental pots.

Parameter	**Unit**	**Properties**
Soil texture	—	Clay:Sandy (2:1)
pH	—	7.2
Organic matter	g/kg	1.8
Total nitrogen (N)	g/kg	0.12
Available phosphorus (P)	mg/kg	15.5
Available potassium (K)	mg/kg	120

Table 2. The analysis of *Bacillus subtilis* extract

Parameter	**Unit**	**Properties**
Viable cell count	CFU/mL	1.5 × 10^8
pH	—	7.2
Viscosity	cP (centipoise)	1.1
Protein concentration	mg/mL	2.5
Antiviral activity	% inhibition	75%
Enzyme activity	U/mL	50

Planting and Treatments*:*

Seeds of faba bean (*Vicia faba* L.) cultivar Giza 716 were obtained from the Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation, Egypt. All agronomic practices and pre-sowing treatments were carried out in accordance with the official recommendations of the Egyptian Ministry of Agriculture. The seeds were certified and treated prior to sowing following standard procedures to ensure optimal germination and plant health.

Faba bean seeds were planted during the two seasons in plastic pots. Sowing took place in the middle of October of each season. Pots with a diameter of 30 cm were used, filled with a soil mixture of clay and sand at a ratio of 2:1 to provide suitable growing conditions. Three plants were grown in each pot, and all pots were maintained under uniform irrigation and agricultural practices to ensure consistent growth and accurate evaluation of experimental treatments.

Layout of the Experiments:

T*he* experiment was conducted using a randomized complete block design (RCBD) in three replicates, with foliar application of *B. subtilis* at concentrations of 0.5, 1.0, 1.5, and 2.0 g/L, along with a control treatment in both seasons with the treatments as follows: Healthy Control, Infected Control, Bacteria-only Control, Pre-infection @ 0.5 g/L, Pre-infection @ 1 g/L, Pre-infection @ 1.5 g/L, Pre-infection @ 2 g/L, Post infection @ 0.5 g/L, Post -infection @ 1 g/L, Post infection @ 1.5 g/L, and Post- infection @ 2 g/L) as shown in Figure 2.

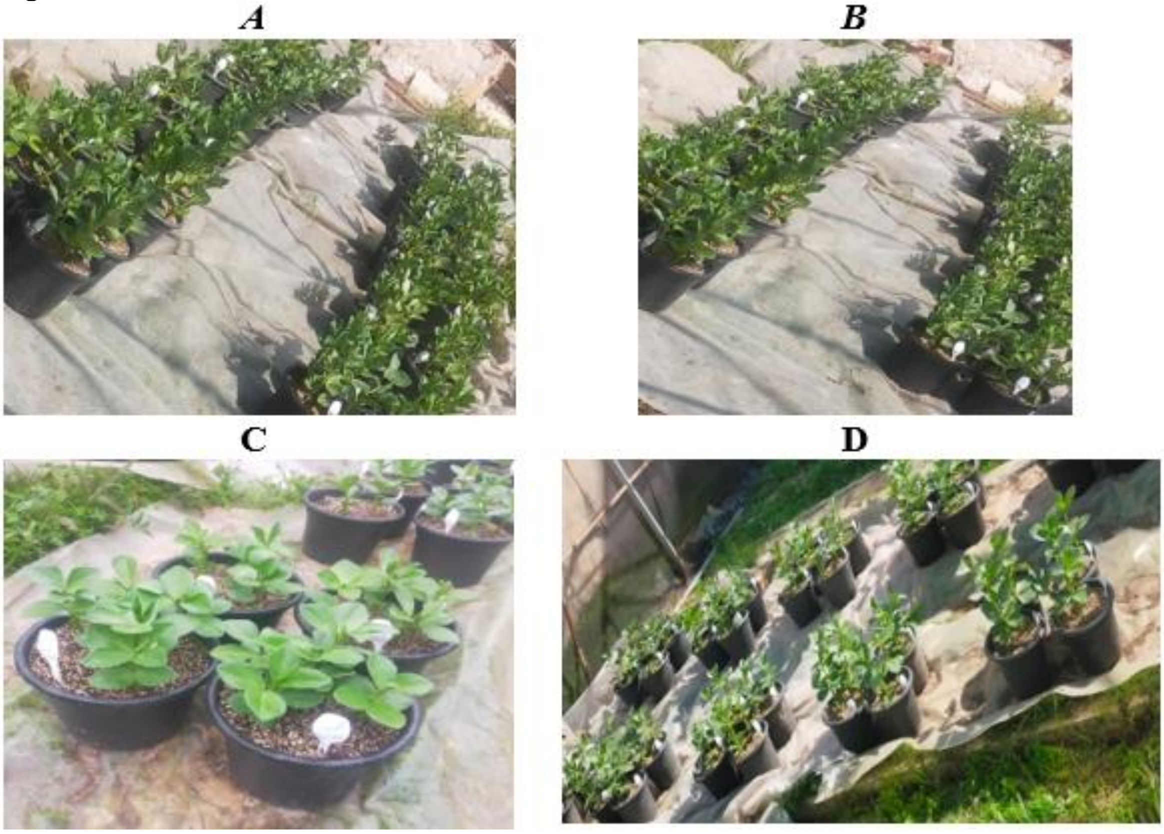

Fig. 2. A, B, C and D show the Layout of the experiment.

Viral Infection:

The infection is transmitted from infected plants to healthy plants mechanically by taking a portion of the infected plant, extracting its sap, and applying it to the healthy plants using a paintbrush. The virus then enters the leaf tissues through tiny wounds or natural openings such as stomata. Once, the virus spreads within the plant cells, causing infection and symptoms on the leaves, including deformities, yellowing, and spotting.

Viral infection was carried out 21 days after sowing. Foliar spraying with *Bacillus* was applied to faba bean plants for 48 hours pre-infection in the preventive treatments (pre-infection), and in the therapeutic treatments (post-infection), foliar spraying was applied 24 hours after the appearance of viral infection symptoms, using the same rates mentioned above.

Preparation Method of Viral Inoculum*:*

A sample of faba bean plants infected with Bean Yellow Mosaic Virus (BYMV) was obtained from the Plant Pathology Department, Sabaheya Station, Alexandria, Agricultural Research Center. The infected leaves were ground in 0.1 M phosphate buffer (pH = 7.0) at a ratio of 1:10 (w/v). The extract was mixed with carborundum powder (600 mesh) to facilitate mechanical inoculation. The resulting sap was applied to the leaves of healthy plants using a fine paintbrush to ensure uniform infection (Fig, 3).

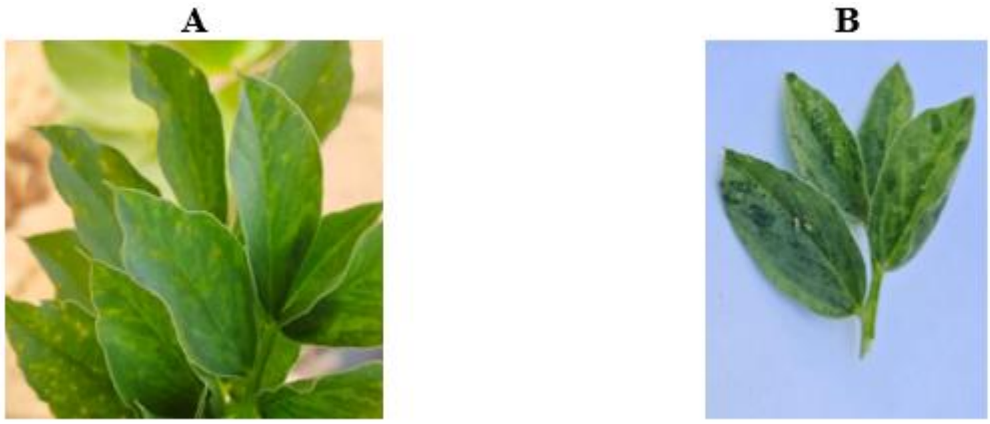

Fig. 3. A and B show the bean yellow virus moasic (BYMV) symptoms.

Detection of Viral Infections:

ELISA (Enzyme-Linked Immunosorbent Assay) was used for the rapid and accurate detection of viral infections in faba bean leaf samples. This technique involves capturing viral antigens present in the plant sap by specific antibodies coated on a microplate. After binding, an enzyme-linked secondary antibody is added, which produces a color change upon reacting with a substrate. The intensity of this color correlates with the amount of virus in the sample, allowing for sensitive and specific virus detection in a relatively short time (Clark and Adams, 1977) as shown in Figure 4.

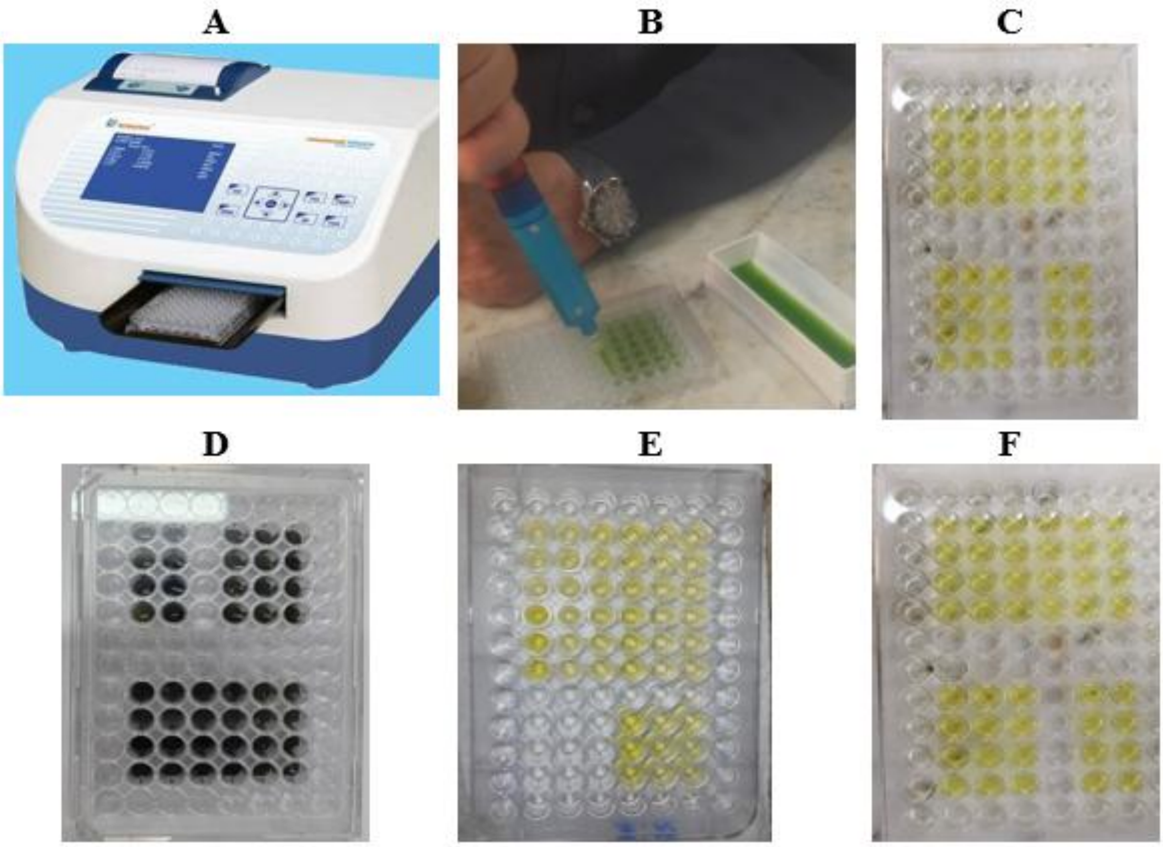

Fig. 4. A, B, C, D, E, and F show the ElISA processes.

Growth Characteristics:

Vegetative samples were collected during the active vegetative growth stage, specifically 60 days after planting in both seasons. This timing was chosen to evaluate the effects of the different treatments on vegetative traits such as plant height (cm), chlorophyll content (SPAD), no. of leaves/plant , number of branches/plant, number of pods/plant and pods weight (g)/plant. Where, the chlorophyll content (SPAD unit) in the leaves was also determined as an indicator of photosynthetic activity and overall plant health.

Statistical Analysis*:*

All collected data were subjected to analysis of variance according to Gomez and Gomez (1984). Statistical analysis was performed using analysis of variance techniques using CoStat computer software package (CoStat, 2005). The least significant difference (LSD at 0.05) was used to compare the treatment means.

RESULTS AND DISCUSSION

A-Use ELSIA For Early Detection of Viral Infections:

Table (3) illustrates the impact of foliar application of *Bacillus subtilis* on disease severity index in faba bean plants infected with Bean Yellow Mosaic Virus (BYMV) over the 2024 and 2025 seasons. The disease severity index is a direct measure of symptom expression, where lower values indicate better plant health and greater resistance to viral infection. The infected control (Treatment 2) recorded the highest severity values in both seasons (1.166 in 2024 and 1.494, respectively in 2025), indicating that BYMV caused severe disease symptoms in untreated plants. This confirms the aggressive nature of the virus and the absence of any defense mechanism when no biocontrol is applied (Bubici *et al*., 2019).

On the other hand, the bacteria-only treatment (Treatment 3), where *Bacillus subtilis* was applied in the absence of viral inoculation, resulted in the lowest disease severity values (0.504 and 0.673), even lower than the healthy control (Treatment 1). This suggests that *B. subtilis* may enhance plant immunity and reduce background stress responses even under non-infected conditions. This agrees with previous studies that emphasized the serious damage viruses cause to legume crops (Bragard *et al*., 2013; Hull, 2014). In contrast, the healthy control and the bacteria-only control (in which *Bacillus subtilis* was applied in the absence of viral infection) resulted in the most favorable plant responses, indicating the role of this beneficial bacterium in enhancing plant health and vigor (Kloepper *et al*., 2004; Glick, 2012).

The preventive treatments (Treatments 4 to 7), in which *Bacillus subtilis* was sprayed before BYMV infection, showed remarkable reductions in disease severity, especially at higher concentrations. Notably, the 2 g/L treatment (Treatment 7) recorded 0.546 in 2024 and 0.732 in 2025, marking the most effective treatment among all. This supports the hypothesis that *B. subtilis* induces systemic resistance in plants when applied before virus attack, preparing the plant's defense system for a faster and stronger response (Hashem *et al*., 2019). This suggests that *B. subtilis* is most beneficial when used as a protective agent prior to infection, in agreement with the concept of induced systemic resistance triggered by plant-growth-promoting rhizobacteria (PGPR) (Ongena and Jacques, 2008).

In contrast, the post-infection treatments (Treatments 8 to 11) were relatively less effective but still showed significant reductions in disease severity compared to the infected control. The best performance among post-infection treatments was recorded at 2 g/L (Treatment 11), with severity values of 0.623 and 0.773, indicating that B. subtilis can still play a therapeutic role, albeit less pronounced than its preventive effect. Again, lower doses were more effective, implying that over-application may not necessarily enhance resistance and may even reduce the bacterium's efficacy (Pal and McSpadden Gardener, 2006; Raaijmakers *et al*., 2009).

The statistical significance of differences between treatments (LSD = 0.178 in 2024 and 0.199 in 2025) reinforces that timing and concentration are critical factors in optimizing the antiviral action of Bacillus subtilis. Overall, these results clearly demonstrate that preventive foliar application, especially at 2 g/L, is the most effective approach to reduce BYMV-induced symptoms and support plant health.

The absorbance values measured using a spectrophotometer are directly proportional to the concentration of the virus in the sample (Clark and Adams, 1977). Thus, higher absorbance values indicate higher levels of viral infection or associated viral proteins. These findings are supported by Bhattacharyya and Jha (2012), who reported the antiviral and ISR-inducing capacity of *Bacillus* species, and Elsharkawy *et al.* (2012), who demonstrated the suppression of viral infection by *B. subtilis* in *Arabidopsis*. Additionally, Kloepper *et al.* (2004) and Ryu *et al.* (2003) highlighted that *Bacillus* spp. enhance plant immunity through signaling pathways and production of bioactive compounds. The significant differences between treatments (as shown by LSD values) emphasize the critical role of timing and concentration in achieving optimal biocontrol performance using beneficial bacteria.

Table 3. ELISA analysis of viral infection (BYMV) in faba bean under different treatments of *Bacillus subtills* in both seasons.

Treatment		**Season**	
		2024	**2025**
1	Healthy Control	0.671	0.897
2	Infected Control	1.166	1.494
3	Bacteria-only Control	0.504	0.673
4	Pre- infection @ 0.5 g/L	0.715	0.871
5	Pre- infection @ 1 g/L	0.665	0.888
6	Pre- infection @ 1.5 g/L	0.632	0.847
7	Pre- infection @ 2 g/L	0.546	0.732
8	Post infection @ 0.5 g/L	0.873	0.937
9	Post infection @ 1 g/L	0.704	0.937
10	Post infection @ 1.5 g/L	0.710	0.885
11	Post infection @ 2 g/L	0.623	0.773
LSD at 0.05		0.178	0.199

B-Effect of foliar application of *Bacillus subtilis* on the Vegetative Growth of Faba Bean Under Viral Infection (BYMV):

The results summarized in Table 4 demonstrate the significant effects of foliar application of *Bacillus subtilis* extract on the vegetative growth and yield-related attributes of faba bean plants under Bean Yellow Mosaic Virus (BYMV) infection across the 2024 and 2025 seasons.

Plants infected with BYMV without any treatment showed the greatest reduction in plant height, chlorophyll content, number of leaves, branches, pods per plant, and pod weight, compared to the healthy control. This confirms the deleterious impact of viral infection on plant physiology and yield potential, in line with earlier findings that viral pathogens disrupt photosynthesis, nutrient uptake, and hormonal balance (Hull, 2014; Bragard *et al*., 2013). Conversely, the bacteria-only control (plants treated with *Bacillus subtilis* without virus infection) exhibited significantly enhanced performance in most measured parameters, surpassing even the healthy control in chlorophyll content, leaf number, and pod weight. These results support previous reports that *B. subtilis* functions as a plant growth-promoting *rhizobacterium* (PGPR), enhancing photosynthetic efficiency and biomass accumulation (Kloepper *et al*., 2004; Glick, 2012).

The preventive treatments, where *B. subtilis* was applied before virus inoculation, showed notable improvements over the infected control, particularly at concentrations of 1.5 and 2 g/L. These treatments maintained higher plant height, leaf number, and pod productivity, suggesting the induction of systemic resistance mechanisms that mitigated viral damage. This aligns with studies indicating that PGPR like *B. subtilis* can activate plant defense pathways before pathogen attack (Ongena and Jacques, 2008; Radhakrishnan *et al*., 2017). On the other hand, the therapeutic treatments (applied after infection) also led to performance improvements compared to the infected control, but to a lesser extent than the preventive ones. Moderate concentrations (1–1.5 g/L) were more effective than lower or higher doses, pointing to a dose-dependent effect in modulating post-infection responses. This finding supports the concept that biocontrol agents are generally more effective as preventative tools than as curative interventions (Pal and McSpadden Gardener, 2006; Raaijmakers *et al*., 2009).

This aligns with findings by Shafi *et al*. (2017), who reported that viral infections often lead to impaired photosynthesis and stunted growth due to oxidative stress and disruption of cellular processes. This is consistent with the study by Bhattacharyya and Jha (2012), who documented that *B. subtilis* acts as a plant growth-promoting rhizobacterium (PGPR), capable of producing phytohormones (like auxins and cytokinins), solubilizing nutrients, and enhancing chlorophyll biosynthesis. Moreover, Kloepper *et al.* (2004) and Rabileh (2023) who reported similar results.

Overall, the study highlights the dual functionality of *Bacillus subtilis* as both a growth enhancer and a biological defense agent. The best results were obtained under preventive application at moderate-to-high concentrations, reinforcing the practical value of this bacterium in integrated viral disease management for legumes.

Table 4. Plant attributes of faba bean as affected by foliar application of *Bacillus subtilis* extract under BYMV infection in both seasons.

	Treatments	Plant height (cm)		Chlorophyll content (SPAD)		No. of leaves/plant		No. of branches/plant		No. of Pods/plant		Pods weight (g)/plant	
		Seasons											
		2024	2025	2024	2025	2024	2025	2024	2025	2024	2025	2024	2025
1	Healthy Control	62.00	74.75	35.50	30.20	62.50	57.00	5.00	3.00	5.75	4.75	3.63	3.43
2	Infected Control	46.25	58.25	26.23	24.25	48.75	42.75	3.75	6.00	3.00	2.00	2.90	2.68
3	Bacteria-only Control	63.25	77.00	51.60	42.85	75.00	70.00	5.75	5.50	6.00	5.00	4.25	4.15
4	Pre- infection @ 0.5 g/L	56.00	62.75	30.75	32.63	72.25	67.00	3.75	6.00	5.50	5.75	3.75	4.00
5	Pre- infection @ 1 g/L	60.00	75.00	39.75	30.38	86.00	80.00	5.25	6.50	6.00	5.00	3.45	3.23
6	Pre- infection @ 1.5 g/L	69.50	84.50	41.75	32.65	93.50	87.50	6.75	5.25	6.50	5.50	4.38	4.38
7	Pre- infection @ 2 g/L	83.50	86.38	44.13	37.88	83.50	81.75	6.50	4.50	5.25	4.25	4.85	4.75
8	Post infection @ 0.5 g/L	47.50	50.00	26.50	26.50	61.00	63.50	5.00	5.50	4.50	4.25	4.25	3.75
9	Post infection @ 1 g/L	67.25	78.25	37.60	32.00	68.75	62.75	5.50	6.50	5.50	4.50	4.10	3.08
10	Post infection @ 1.5 g/L	63.75	71.50	35.48	32.13	70.25	65.00	5.75	5.25	6.50	5.50	4.25	3.40
11	Post infection @ 2 g/L	68.75	77.50	32.93	33.25	67.25	61.25	6.25	5.75	5.25	4.50	5.15	4.85
	LSD at 0.05	**9.64**	**7.98**	**7.83**	**7.68**	**13.69**	**13.66**	**1.78**	**1.79**	**1.13**	**0.96**	**0.93**	**0.89**

Conclusion:

Overall, the application of *B. subtilis* extract, especially @ the rate of 1.5 or 2 g/L pre infection, significantly enhanced plant growth and yield attributes of faba bean under viral stress like BYMV. The biocontrol agent proved effective in both preventive and curative roles, with superior efficacy in the former. These findings support the potential of *B. subtilis* as a sustainable, eco-friendly alternative to chemical protection in legume production.

Declarations:

Ethical Approval: This study did not involve any live animals. It was based solely on storage-based laboratory analysis of plant by-products.

Conflict of interest: The authors declare no conflict of interest.

Author's Contributions: I hereby verify that the authors mentioned on the title page has Contributed significantly to the idea and planning of the research, has carefully read the work, attested to the veracity and correctness of the data and its interpretation, and has given their approval for submission.

Availability of Data and Materials: All data generated or analyzed during this study are included in this article.

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ARABIC SUMMARY

تقييم بكتيريا باسيلس سبتليس كعامل مكافحة حيوية ضد الممرضات الفيروسية في الفول البلدي

إبراهيم ناجي نوح[1] ، أحمد حسين زيد[2] ، حسني علي يونس[2]، منى محمد يسري جابر[1]

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يُعد الفول البلدي من المحاصيل البقولية الأساسية في مصر، حيث يُزرع على نطاق واسع كمصدر رئيسي للبروتين النباتي. إلا أنه غالبًا ما يتعرض للإصابة بعدة فيروسات تؤثر سلبًا على نموه وإنتاجيته، مما يستدعي البحث عن وسائل فعالة لمكافحتها، خاصة باستخدام طرق المكافحة الحيوية كبديل آمن وصديق للبيئة. لذا تم إجراء تجربتين حقليتين بكلية الزراعة (سبا باشا)، جامعة الإسكندرية، مصر، خلال موسمي 2024/2023 و2025/2024، بهدف تقييم فعالية بكتيريا *Bacillus subtilis* كعامل مكافحة حيوية في الحد من حدوث وشدة الإصابات الفيروسية في نمو نباتات الفول البلدي. وقد نُفذت التجربة خلال موسمين متتاليين باستخدام تصميم القطاعات العشوائية الكاملة (RCBD) بثلاث مكررات، حيث تم رش بكتيريا *Bacillus subtilis* على الأوراق بتراكيز (0.5، 1.0، 1.5، و2.0 جم/لتر)، بالإضافة إلى معاملة كنترول صحى وكنترول مصاب ومعاملة بالبكتيريا فقط) في كلا الموسمين.

أظهرت النتائج أن المعاملة ببكتيريا *Bacillus subtilis* قبل الإصابة الفيروسية (BYMV) بتركيز 1.5 جم/لتر أدت إلى أعلى القيم في جميع الصفات المدروسة، حيث بلغ متوسط طول النبات 84.5 سم في الموسم الثاني، وعدد القرون 93.5 قرن في الموسم الأول، ووزن القرون 4.38 جم في كلا الموسمين. ويشير ذلك إلى أن استخدام البكتيريا قبل الإصابة يعزز قدرة النبات على النمو والتطور ومقاومة الفيروس. أما المعاملة بتركيز 2 جم/لتر قبل الإصابة فقد أظهرت تحسنًا في الصفات لكنها كانت أقل من معاملة 1.5 أو 2 جم/لتر في بعض المؤشرات مثل عدد ووزن القرون، ما قد يشير إلى أن التركيز الأعلى ليس بالضرورة الأفضل دائمًا، وربما يسبب إجهادًا بسيطًا للنبات. في المقابل، سجلت النباتات المصابة غير المعاملة (الكنترول المصاب) أدنى القيم في جميع الصفات، حيث انخفضت نسبة الكلوروفيل وعدد الأوراق والأفرع، مما يؤكد التأثير الضار للإصابة الفيروسية على نشاط النبات الحيوي وإنتاجه. كما أن المعاملة بالبكتيريا فقط دون وجود فيروس (البكتيريا فقط) أظهرت أداءً جيدًا، مما يدل على قدرة *Bacillus subtilis* على تعزيز النمو حتى في غياب مسببات المرض. أما المعاملات التي تم فيها رش المستخلص بعد حدوث الإصابة الفيروسية، فقد أظهرت تحسنًا ملحوظًا مقارنة بالكنترول المصاب.

التوصية:

يوصى البحث باستخدام مستخلص *Bacillus subtilis*، خاصة بتركيز 1.5 أو 2 جم/لتر قبل الإصابة، له تأثير إيجابي في تحسين نمو وإنتاج الفول البلدي وتقليل الأضرار الناتجة عن الإصابة الفيروسية بـ (BYMV)، مما يعكس أهمية هذا المستخلص كعامل حيوي واعد في الزراعة المستدامة. كما تُعد تقنية ELISA أداة حساسة وموثوقة تُستخدم للكشف عن الإصابات الفيروسية في النباتات من خلال قياس تركيز البروتينات الفيروسية. كما تساعد في تقييم مدى فعالية المعاملات الحيوية في تقليل وجود الفيروس.