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Influence of Vermicompost and *Bacillus subtilis* on the Vegetative Characteristics of Local Grapevine (*Vitis vinifera* L.) Seedlings

Wasan Waleed Ahmed

Northern Technical University, Agricultural Technical College, Department of Plant Production Technologies, Iraq

Corresponding Author: Wasan Waleed Ahmed: wsnalobaidy@ntu.edu.iq

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ABSTRACT

The study was carried out in 2025 on a vine nursery in a greenhouse at the Northern Technical University, Technical Agricultural College, Iraq, where the impact of the use of vermicompost and *Bacillus subtilis* on the vegetative growth of local 'Zark' grapevines (*Vitis vinifera* L.) transplants was evaluated. The experimental design used for this research is a factorial experiment with a Randomized Complete Block Design (RCBD), where the treatments include the use of vermicompost at four different levels (0, 100, 200, 300 g/plant) along with *Bacillus subtilis* suspension at two different concentrations (0, 10 ml. plant^{-1}). The growth parameters were shoot length, leaf number, leaf area, branch number, stem diameter, and shoot dry weight. The findings clearly show the effectiveness of the application of vermicompost in stimulating all vegetative growth parameters, particularly when applied at the highest concentration of 300 g. plant^{-1}. In the same way, the inoculation of *Bacillus subtilis* positively affected growth performance relative to those without inoculation. When combining 300 g. plant^{-1} vermicompost and 10 mL/plant *Bacillus subtilis*, the maximum positive results were observed, including height of the main stem, leaf number, leaf area, branch number, stem diameter, and shoot dry weight being 63.0 cm, 20, 66.0 cm^2, 6, 9.2 mm, and 9.8 g, respectively. In comparison, the lowest results were found in the case of the untreated control.

Keywords: biofertilizer application; organic soil amendments; *Bacillus subtilis*.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the economically most important fruits produced across the globe, and the significance of this crop in the Arab world, especially in Iraq, dates back several thousands of years, with local cultivars being grown in places such as Nineveh, Dohuk, and Sulaymaniyah. It should be noted that the nursery phase is believed to be a crucial stage in viticulture since the quality of the transplant directly affects future yields, fruit characteristics, and even success in establishment (1). Grapevine is regarded as one of the most valuable fruit products produced around the globe, and it has been grown in Iraq for a long period of time. There are an estimated 70 grape varieties in Iraq; grape cultivation is common, particularly concentrated in the northern areas of the country (2). History proves that grapes were first domesticated some 6,000 to 8,000 years ago in an area situated between the Black and Caspian Seas. After that, cultivation began expanding into Asian territories and towards the Mediterranean region. The climate areas situated between 34° and 45°N latitude and 31–38°S can be referred to as the most favorable ones for grape growing (3). Based on the (4) statistics, currently, there are approximately 6,730,179 hectares under grapes with an annual yield amounting to In Iraq, a total of 74,942,572 tons was

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recorded, grapes cover an locality of about covering 22,322 ha yielding 384,984.15 tons per year. Grape berries are distinguished not only by their nutritional value but also by medicinal benefits, as they contain bioactive components such as phenolic compounds, flavonoids, aromatic acids, proanthocyanidins, stilbenoids, and tartaric acid, which constitute the main phytochemicals of grapes (5). Nevertheless, nowadays the production of high-quality grapevine transplants remains dependent on mineral fertilizers, entailing economic expenses and serious negative impacts on the environment, namely soil acidification, leaching of nutrients, and disturbances in the soil microbial community (6).

During the past few decades, organic amendments and Plant Growth-Promoting Rhizobacteria (PGPR) have been applied as sustainable input materials in crop production. Of organic amendments, vermicomposts, which result from the degradation of organic matter by earthworms, have gained much attention due to the unique combination of physicochemical and biological characteristics that make them an excellent amendment. Vermicompost consists of highly concentrated humus, organic matter, minerals, and beneficial microorganisms in plant-available forms, all of which promote plant growth through multiple mechanisms (7, 8). The use of chemical fertilizers and pesticides has greatly increased agricultural production in the recent past. However, the excessive utilization of such chemicals is now known to be responsible for many environmental problems such as soil degradation and pollution, and has harmful effects on human health. This has resulted in the recent focus on sustainable agriculture methods, organic farming, and the application of bio-fertilizers. Beneficial microorganisms in bio-fertilizers improve soil fertility and promote the growth of plants without harming the environment. Organic fertilizers not only improve soil conditions but are also rich in microorganisms. There is increasing evidence suggesting that bio-fertilizer and organic fertilizer utilization improves the vegetative growth, yield, and quality of fruits produced by several fruit crops. It has also been proposed that combined application of organic fertilizers and beneficial microorganisms could enhance growth in plants and reduce the requirement for synthetic fertilizers. These methods of enhancing plant growth are seen as important steps towards sustainable agricultural production (9). Multiple studies have demonstrated increased shoot growth, root development, and total biomass accumulation in horticultural transplants with vermicompost amendments in studies conducted by (8, 10).

The Bacillus subtilis bacterium, which is gram-positive and an endospore-forming soil bacterium, can be considered one of the most widely investigated plant growth-promoting rhizobacteria (PGPR). The ability of this bacterium to stimulate plant growth stems from multiple factors, such as phosphate solubilization, synthesis of IAA, and other phytohormones, volatile metabolites like 2,3-butanediol, and induction of systemic resistance against a broad spectrum of pathogens (11, 12). Studies have shown that application of *Bacillus subtilis* to nursery stock results in faster root growth and increased chlorophyll accumulation in the leaves of various types of fruit trees, including grapevines (13, 14).

There is a growing need for environmentally friendly practices in the implementation of sustainable agriculture, such as using biofertilizers. As their name implies, biofertilizers comprise useful microorganisms, including bacteria, fungi, and algae, that enhance soil fertility and plant productivity by providing nutrients and other biological functions. Compared to chemical fertilizers, biofertilizers are environmentally friendly and biodegradable, leading to reduced soil and water pollution. Furthermore, they improve agricultural productivity and the utilization of nutrients while maintaining healthy soils in stressful conditions such as drought. Some recent studies indicate the importance of biofertilizers in promoting sustainable production

practices in agriculture while minimizing the harmful environmental effects of chemical fertilizers (15).

According to the hypothesis, the mixture of organic materials and PGPRs may exert a synergistic effect, as the organic material in vermicompost serves as an ideal substrate that promotes bacterial proliferation and activity, and the presence of bacteria leads to the faster breakdown and mineralization of nutrients by organic materials (16). Little information is known about the combined impact of different vermicompost application rates with varying amounts of inoculated B. subtilis on vegetative growth of the local cultivars of grapes in northern Iraq, where semi-arid climatic conditions prevail. This study aims to analyze the impact of graded vermicompost application rates and B. subtilis inoculation on different vegetative growth traits of local grapevine plants grown in the nursery.

Materials and Methodology

Study Site and Plant Material

The investigation took place in the 2025 season in a greenhouse located at the Technical Agricultural College, Northern Technical University, Iraq. This location can be considered an optimal setting to assess the influence of different factors on the response of nursery-stage plants grown under similar environmental conditions. The plant materials were local 'Zark' grapevine (*Vitis vinifera* L.) cuttings. Only healthy, uniform cuttings with one node each were used to avoid any discrepancies in the growth rate. The cuttings served as propagating material to grow the test plants in the greenhouse setting. The use of greenhouse conditions made it possible to control environmental factors and ensure uniform growth conditions within each treatment. This allowed the effects of the proposed treatments to be clearly defined. Plants were grown in the greenhouse to assess the effects of vermicompost and Bacillus subtilis treatment in the nursery phase.

Research Design and Treatment Regime

The experimental work was arranged in a Randomized Complete Block Design (RCBD), where the two experimental factors were: vermicompost factor, Vermicompost application at four rates: V0 = 0 g·plant^{-1} (control), V1 = 100 g·plant^{-1}, V2 = 200 g·plant^{-1}, and V3 = 300 g·plant^{-1}. The vermicompost applied in this study was commercially produced from sheep manure and kitchen waste and characterized by a pH of 6.8, electrical conductivity of 2.1 dS·m^{-1}, total organic carbon of 32.4%, total nitrogen of 2.1%, available phosphorus of 820 mg·kg^{-1}, and available potassium of 1,540 mg·kg^{-1}. The product was thoroughly mixed into the growing medium at the time of container preparation. B. subtilis factor, Bacillus subtilis bacterial suspension at two levels: without B. subtilis = 0 mL.plant^{-1} (control), B. subtilis 10 mL.plant^{-1} = 10 mL.plant^{-1} . "The bacterial suspension contained approximately 1 × 10^8 CFU mL^{-1} and was applied as a soil drench around the root zone at transplanting." The bacterial suspension was prepared from a locally isolated and identified B. subtilis strain. *Bacillus subtilis* bacteria were prepared in the laboratories at the Technical Agricultural College, Northern Technical University. The bacterial isolate was obtained and cultured under laboratory conditions using suitable nutrient media to ensure active growth and sufficient inoculum density for use in the experiment, and cultured in nutrient broth, incubated at 28 °C for 48 hours

Cultural Practices

All experimental units received identical cultural management throughout the study period. Containers were arranged on raised benches under shade net (50% light interception) to simulate nursery conditions. Irrigation was applied uniformly by drip system at 48-hour intervals. No mineral fertilizers were applied to any treatment during the experimental period, so as to isolate the effects of the organic and biological inputs under study. Routine weeding and pest monitoring were conducted on a weekly basis.

Growth Parameters Measured

Six vegetative growth parameters were recorded at 90 days after transplanting. Main stem height (cm) was recorded from the base of the primary shoot to the apical meristem using a graduated ruler. Leaf number was expressed as the total number of fully expanded leaves per plant. Leaf area (cm^2) was measured on the third fully expanded leaf from the apex of each plant using a portable leaf area meter. Branch number was recorded as the total count of lateral shoots exceeding 2 cm in length. Stem diameter (mm) was recorded at a height of 5 cm above the substrate surface using a digital Vernier caliper, while shoot dry weight (g) was measured after oven-drying the aboveground biomass at 70 °C for 72 h to constant mass.

Data Analysis

All data were analyzed using analysis of variance (ANOVA) appropriate for a factorial randomized complete block design (RCBD) with SPSS software version 26.0 (IBM Corp., USA). Differences among treatment means were compared using the Least Significant Difference (LSD) test at the 5% probability level ($P \leq 0.05$). Prior to ANOVA, data normality was assessed using the Shapiro–Wilk test, while homogeneity of variances was evaluated using Levene's test. Results are presented as means ± standard error of the mean (SEM) based on five replicates.

Results and discussion

Height of the main stem

The results presented in the table show a significant effect of vermicompost application, *Bacillus subtilis* inoculation, and their interaction on the shoot length of local grapevine cv. Zark. The results demonstrated a progressive and significant increase in shoot length with increasing vermicompost levels and bacterial inoculation in comparison with the control treatment (0 g vermicompost + without B. subtilis), which recorded the lowest mean shoot length of 29.82 cm, whereas inoculation with B. subtilis alone increased shoot length to 34.22 cm, reflecting a positive individual effect of the bacterium on vegetative growth. The application of 100 g $plant^{-1}$ vermicompost increased shoot length to 36.98 cm in the absence of inoculation and to 42.66 cm under bacterial inoculation. A further increase in vermicompost level to 200 g $plant^{-1}$ resulted in shoot lengths of 46.24 cm without inoculation and 53.74 cm with B. subtilis treatment. The highest values were recorded at 300 g $plant^{-1}$ vermicompost, where shoot length reached 56.14 cm without bacterial inoculation and increased further to 63.60 cm under the combined application of the highest vermicompost level and bacterial inoculation, indicating a clear and significant superiority over all other treatments. The overall means of the studied factors showed that increasing vermicompost levels markedly improved shoot length, rising from 32.02 cm in the control treatment to 59.87 cm at 300 g $plant^{-1}$. In addition, inoculation with Bacillus subtilis increased the overall mean shoot length from 42.30 to 48.56 cm.

Table 01: Effect of Vermicompost and *Bacillus subtilis* on the height of the main stem (cm) of Local Grapevine (*Vitis vinifera* L.) Transplants

Vermicompost	Without *Bacillus subtilis*	*Bacillus subtilis* (10 mL)	Effect of Vermicompost
(0 g)	29.82 h	34.22 g	**32.02 d**
(100 g)	36.98 f	42.66 e	**39.82 c**
(200 g)	46.24 d	53.74 c	**49.99 b**
(300 g)	56.14 b	63.60 a	**59.87 a**
Effect of *Bacillus subtilis*	**42.30 b**	**48.56 a**	

Averages following the same letter do not differ significantly according to Duncan's multiple range test ($P \leq 0.05$).

Vermicompost contains unique characteristics regarding physical, chemical, and biological features, which help achieve greater vegetative growth. On a physical basis, the granulated structure makes the soil better for aeration and moisture retention, thus enhancing roots' respiration and nutrient uptake (17). As an active agent of vermicompost, humic acids enhance nutrient uptake by enhancing the permeability of roots' cellular membranes and stimulating their development along with root hair multiplication (18). Regarding plant growth at the hormonal level, research indicates that the extraction of humic acids from vermicompost is capable of showing auxin and cytokinin action through hormonal biosynthesis pathway modification. Accordingly, (19) found remarkable improvements in the growth of wheat via auxin and cytokinin biosynthesis pathway modulation. Earthworms, together with microorganisms involved in vermicomposting, produce plant growth hormones including auxins, gibberellins, and cytokinins. Moreover, the presence of biologically active auxins, including indole-3-acetic acid (IAA), in the humic acids fraction from vermicompost was established (19).

Bacillus subtilis functions as a PGPR in several ways that relate to the stimulation of elongation in the shoots of plants. The bacterium synthesizes IAA, an example of a secondary metabolite, which causes increased growth and division of cells at meristems (20). It also synthesizes gibberellins, which promote cell elongation in plant elongation zones (21). Moreover, it contributes to phosphorus solubilization by secreting phosphatases and organic acids, converting insoluble soil phosphorus into plant-available forms, thereby supplying phosphorus required for ATP production essential for vegetative growth (22). In addition, ACC deaminase activity reduces ethylene levels, a key growth-inhibiting hormone, thereby alleviating one of the main constraints on vegetative elongation (23). Furthermore, (24) demonstrated that IAA-producing Bacillus species increase the expression of growth-related genes, such as expansins (EXP-1 and EXP-2), along with auxin- and gibberellin-responsive genes, thereby further promoting plant growth.

The highest shoot length value recorded at the combined treatment of 300 g plant^{-1} vermicompost with bacterial inoculation (63.60 cm) confirms a true synergistic interaction between the two factors. Vermicompost provides an optimal soil environment that enhances bacterial activity and proliferation, while also supplying the organic carbon and energy sources required for the production of bacterial metabolites and growth-promoting compounds. Such a synergistic interaction has been established in previous research conducted on cherry tomato plants where vermicompost in combination with Bacillus spp., such as B. subtilis, yielded better vegetative growth attributes than those achieved using the individual treatments (25).

Leaf Number

Table (2) shows that there is an observable significant effect of applying vermicompost, Bacillus subtilis, and their interaction on the number of leaves per transplant. From the study results, there was a definite and gradual increase in the number of leaves per transplant with increasing rates of vermicompost and bacterial inoculation compared to the control. The control treatment (0 g vermicompost + without B. subtilis) recorded the lowest mean leaf number of 7.98 leaves plant^{-1}, whereas inoculation with B. subtilis alone increased the number to 10.00 leaves plant^{-1}, reflecting a positive individual effect of the bacterium on vegetative growth. The application of 100 g plant^{-1} vermicompost increased leaf number to 10.98 leaves plant^{-1} in the absence of inoculation and to 12.84 leaves plant^{-1} under bacterial inoculation. An increase in vermicompost level to 200 g plant^{-1} further enhanced leaf number, reaching 14.20 leaves plant^{-1} without inoculation and 15.88 leaves plant^{-1} with B. subtilis. The highest values were recorded at 300 g plant^{-1} vermicompost, where leaf number reached 16.90 leaves plant^{-1} in

the absence of bacteria and increased to 20.32 leaves plant^{-1} under the combined treatment of the highest vermicompost level and bacterial inoculation, indicating a clear and significant superiority over all other treatments. The overall means of the studied factors showed that vermicompost application markedly increased leaf number from 8.99 leaves plant^{-1} in the control to 18.61 leaves plant^{-1} at 300 g plant^{-1}. In addition, Bacillus subtilis inoculation increased the overall mean leaf number from 12.52 to 14.76 leaves plant^{-1}.

Table 02: Effect of Vermicompost and *Bacillus subtilis* on Leaf Number of Local Grapevine (*Vitis vinifera* L.) Transplants

Vermicompost (A)	Without Bacillus subtilis	Bacillus subtilis (10 mL)	Effect of Vermicompost
(0 g)	7.98 h	10.00 g	**8.99 d**
(100 g)	10.98 f	12.84 e	**11.91 c**
(200 g)	14.20 d	15.88 c	**15.04 b**
(300 g)	16.90 b	20.32 a	**18.61 a**
Effect of *Bacillus subtilis*	**12.52 b**	**14.76 a**	

Averages followed by the same letter do not differ significantly according to Duncan's multiple range test ($P \leq 0.05$).

Leaf formation and development require adequate availability of essential nutrients and sufficient plant hormones to activate meristematic cells in leaf buds. Vermicompost provides N, P, and K in forms accessible to plants. Specifically, nitrogen is an important element of nucleic acids and proteins involved in cell division and formation of vegetative parts of plants, particularly leaves (26). Humic acid found in vermicompost regulates plant hormone signal transduction pathways; therefore, humic acid can be used as an activator that can stimulate the production of endogenous auxin and cytokinin and, hence, lateral bud activation and increased leaf production (27). Moreover, vermicompost's physical properties enhance the aeration of the roots, which results in enhanced nutrient uptake and better plant growth.

There are two mechanisms by which Bacillus subtilis activates leaf buds. First, the bacterium promotes phytohormone production, especially cytokinins and auxins. An independent investigation indicated that the B. subtilis SB3 strain produced IAA, cytokinins, gibberellins, and abscisic acid, leading to a substantial the number of leaves increased to 49.66 per plant (28). Second, Bacillus subtilis promotes the availability of phosphorus by dissolving it from organic and inorganic soil compounds, thus allowing DNA synthesis in actively dividing cells of leaf buds.

Leaf Area

According to Table (3), the use of vermicompost, the inoculation of Bacillus subtilis, and their interaction significantly affected the leaf area of the local grapevine variety cv. Zark transplants. According to the data obtained, there was an obvious continuous growth in the leaf area with the increase in vermicompost levels and bacterial inoculation in comparison with the control. The control treatment, that is, the addition of 0 g of vermicompost + without Bacillus subtilis, registered the lowest average leaf area, which reached 29.52 cm². The inoculation with *Bacillus subtilis* resulted in 34.74 cm² of leaf area and proved the individual influence of the bacterium on leaf properties. This improvement continued with increasing vermicompost level to 200 g plant^{-1}, where leaf area reached 47.82 cm² without

inoculation and 52.76 cm² with B. subtilis. The highest values were recorded at 300 g plant⁻¹ vermicompost, reaching 56.66 cm² without bacterial inoculation and increasing to 65.98 cm² under the combined application of the highest vermicompost level and bacterial inoculation, indicating a clear and significant superiority over all other treatments. The overall means of the studied factors showed that vermicompost application increased leaf area markedly from 32.13 cm² in the control to 61.32 cm² at 300 g plant⁻¹. In addition, inoculation with Bacillus subtilis increased the overall mean leaf area from 43.01 to 49.07 cm².

Table 03: Effect of Vermicompost and Bacillus subtilis on Leaf Area of Local Grapevine (*Vitis vinifera* L.) Transplants

Vermicompost	Without Bacillus subtilis	Bacillus subtilis (10 mL)	Effect of Vermicompost
(0 g)	29.52 h	34.74 g	**32.13 d**
(100 g)	38.02 f	42.80 e	**40.41 c**
(200 g)	47.82 d	52.76 c	**50.29 b**
(300 g)	56.66 b	65.98 a	**61.32 a**
Effect of *Bacillus subtilis*	**43.01 b**	**49.07 a**	

Averages following the same letter do not differ significantly according to Duncan's multiple range test (P ≤ 0.05).

The adequate and readily available supply of macro- and micronutrients is a fundamental factor explaining the observed increase in leaf area. Available nitrogen enhances chlorophyll synthesis, which in turn improves photosynthetic efficiency and the accumulation of organic compounds required for leaf expansion. Humic acids also play an important role by activating the plasma membrane H^+-ATPase, which is responsible for acidifying the cell wall environment and loosening its structure, thereby facilitating cell wall expansion and leaf enlargement (29).

Moreover, it was found in another research carried out in 2024 on grapes (Vitis vinifera cv. Touriga Franca) using vermicompost made from pruning residues of grapevines in a Mediterranean environment that there was substantial improvement in physiological and biochemical performance because of an increase in vegetative growth development (30).

The secretion of IAA by B. subtilis plays a key role in increasing leaf area by enabling the plant to cell walls less stiff and causing cells to enlarge, besides causing cell division in the growing leaves (31). It is mentioned by (32) that auxins and cytokinins released by Bacillus spp. expand root surface area and, as a consequence, improve nutrient absorption by the roots; this, in turn, results in expansion of the leaves. Furthermore, cytokinin produced by B. subtilis supports cell division within the leaves, resulting in greater total leaf area (21).

Branch Number

Table (4) data indicate a significant effect of vermicompost application, Bacillus subtilis inoculation, and their interaction on the number of lateral shoots in local grapevine cv. Zark transplants. The results showed a clear increase in shoot number with increasing vermicompost levels and bacterial inoculation relative to the control treatment. The control treatment (0 g vermicompost + without B. subtilis) recorded the lowest mean number of shoots (2.08 shoots plant⁻¹). Application of 100 g plant⁻¹ vermicompost without bacterial inoculation increased shoot number to 3.06 shoots plant⁻¹, and it

further increased to 3.96 and 4.96 shoots plant^{-1} at 200 and 300 g plant^{-1}, respectively, indicating a progressive response with increasing vermicompost levels. Inoculation with B. subtilis alone (without vermicompost) increased shoot number to 2.76 shoots plant^{-1} compared with the control, reflecting a positive individual effect of the bacterium in stimulating lateral branching. The presence of vermicompost in conjunction with the application caused a significant rise, with 3.92, 5.00, and 5.98 shoots per plant recorded at doses of 100, 200, and 300 g of vermicompost per plant, respectively. The maximum number of lateral shoots was produced in the interactive treatment (300 g of vermicompost + 10 mL of B. subtilis), which resulted in the production of 5.98 shoots per plant. The overall means of the studied factors showed that vermicompost increased shoot number from 2.42 to 5.47 shoots plant^{-1} at the highest level, while B. subtilis inoculation increased the overall mean from 3.52 to 4.42 shoots plant^{-1}.

Table 04: Effect of Vermicompost and *Bacillus subtilis* on Branch Number of Local Grapevine (*Vitis vinifera* L.) Transplants

Vermicompost	Without Bacillus subtilis	Bacillus subtilis (10 mL)	Effect of Vermicompost
(0 g)	2.08 f	2.76 e	**2.42 d**
(100 g)	3.06 d	3.92 c	**3.49 c**
(200 g)	3.96 c	5.00 b	**4.48 b**
(300 g)	4.96 b	5.98 a	**5.47 a**
Effect of *Bacillus subtilis*	**3.52 b**	**4.42 a**	

Averages following the same letter do not differ significantly according to Duncan's multiple range test ($P \leq 0.05$).

Apical dominance regulates the formation of lateral shoots; a high auxin level produced by the apical meristem suppresses the activity of lateral buds, whereas cytokinins exert an opposite effect by promoting their activation. Thus, the availability of appropriate amounts of external auxin (from vermicompost and bacteria), along with cytokinins, leads to the establishment of a hormonal balance, breaking the apical dominance and initiating shoot growth. In addition, since ethylene regulates the process of branching at the lateral bud level, changes in its concentration will affect the number of shoots (23).

The contribution of vermicompost to lateral branch formation relates to the provision of phytohormones such as auxins, cytokinins, gibberellins, and humates, contributing to optimal hormonal balance, leading to the release of lateral buds from apical dominance (8). At the same time, an appropriate amount of nitrogen and potassium stimulates carbohydrate synthesis in stem knots, which provide energy to lateral buds to overcome dormancy and grow.

Moreover, *Bacillus subtilis* facilitates the process of lateral branch formation by decreasing ethylene levels through ACC deaminase (10). According to the results of the experimental work with wild chickpeas, the B. subtilis strain, capable of producing IAA, cytokinins, and gibberellins, significantly increased the shoot number to 15.66 per plant, while the number was 0.75 shoots in the control group (28). Besides, Bacillus subtilis produces siderophores, which are responsible for iron solubilization and increasing its bioavailability to plants. Iron is a key component of redox enzymes involved in cellular metabolism and is essential for

activating lateral bud cells and supporting their growth.

Stem Diameter

As shown in Table 5, there was a highly significant effect of the application of vermicompost, Bacillus subtilis inoculation, and their interaction on the stem diameter of local grapevine cv. Zark transplants. In this regard, it is noticed that stem diameter was significantly enhanced by increasing the quantity of vermicompost and bacterial inoculation compared with the control treatment. In regard to the stem diameter results, the control treatment (0 g vermicompost + without B. subtilis) had the lowest mean (4.31 mm). On the other hand, increasing the amount of vermicompost in plants (without B. subtilis inoculation) caused a progressive increase in the stem diameter, which ranged from 5.33, 6.69, and 7.94 mm. As for the stem diameter of inoculation B. subtilis in the absence of vermicompost, the mean value was 5.09 mm, which is higher than the control value. However, the interaction between these two factors (vermicompost + B. subtilis inoculation) resulted in the highest values, where the mean values increased to 6.38, 7.92, and 9.24 mm, respectively. The interaction between the two factors resulted in the highest value of stem diameter (9.24 mm). In regard to the mean values of the studied factors, vermicompost increased the stem diameter from 4.70 to 8.59 mm, while B. subtilis inoculation increased the overall mean from 6.07 to 7.16 mm.

Table 05: Effect of Vermicompost and Bacillus subtilis on Stem Diameter (mm) of Local Grapevine (*Vitis vinifera* L.) Transplants

Vermicompost	Without Bacillus subtilis	Bacillus subtilis (10 mL)	Effect of Vermicompost
(0 g)	4.31 g	5.09 f	**4.70 d**
(100 g)	5.33 e	6.38 d	**5.85 c**
(200 g)	6.69 c	7.92 b	**7.30 b**
(300 g)	7.94 b	9.24 a	**8.59 a**
Effect of *Bacillus subtilis*	**6.07 b**	**7.16 a**	

Averages following the same letter do not differ significantly according to Duncan's multi-range test (P ≤ 0.05).

Vermicompost supplies essential nutrients required for vascular tissue development. Calcium contributes to cell wall formation through the deposition of calcium pectate in the middle lamella, while sufficient potassium regulates turgor pressure, which is essential for cell expansion in actively dividing cambial cells. A recent study confirmed that vermicompost application significantly improves growth substrate structure and soil properties, which positively reflects on plant growth, including stem diameter. Humic acids present in vermicompost also play a key role in stimulating vascular cambium activity, as they have been shown to upregulate genes involved in auxin biosynthesis, a major regulator of cambial activity and stem thickening (19).

Bacillus subtilis enhances stem thickening through multiple mechanisms. The IAA it produces stimulates cell division in the vascular cambium and increases procambial activity responsible for the formation of xylem and phloem tissues, both of which contribute to stem diameter increase (20). (24) further demonstrated that IAA-producing Bacillus spp. enhance the expression of expansin genes (EXP-1 and EXP-2), which loosen cell walls and facilitate cell expansion, thereby contributing to increased stem diameter.

In addition, phosphate solubilization improves the plant's energy supply (ATP), which is essential for wood formation and cellular expansion in the stem. ACC deaminase activity also reduces ethylene levels in vascular tissues, thereby alleviating one of the major constraints on cambial growth (23).

The large difference between the control treatment (4.31 mm) and the combined treatment at 300 g plant^{-1} (9.24 mm) clearly reflects a strong synergistic effect between vermicompost and *B. subtilis*. In this treatment, vermicompost functions as a nutrient source and provides growth-promoting substances that support cell expansion, while B. subtilis further stimulates cambial activity and vascular tissue formation, resulting in more than a twofold increase in stem diameter compared with the control. This agrees with previous findings that combining organic amendments with plant growth-promoting bacteria produces effects greater than the additive impact of each factor alone (25).

CONCLUSIONS

The results of this study indicate the following conclusions:

1. The application of vermicompost at increasing levels (100, 200, and 300 g plant^{-1}) significantly improved all vegetative growth traits of local grapevine cv. Zark transplants, including Height of the main stem, number of leaves, leaf area, number of branches, and stem diameter, in a dose-dependent manner, with the highest level (300 g plant^{-1}) recording the best performance across all studied parameters.

2. Inoculation with Bacillus subtilis (10 mL plant^{-1}) alone exerted a significant positive effect on all vegetative growth traits compared with the uninoculated control, confirming its role as an effective plant growth-promoting rhizobacterium (PGPR) capable of enhancing vegetative development through phytohormone production, phosphate solubilization, and ACC deaminase activity.

3. The combined application of vermicompost and Bacillus subtilis inoculation produced a synergistic effect that surpassed the individual effects of either treatment alone across all studied traits, indicating a biological complementarity between the organic amendment and the bacterial inoculant in promoting vegetative growth.

4. The interaction treatment of 300 g plant^{-1} vermicompost combined with Bacillus subtilis inoculation recorded the highest values for all studied traits: shoot length (63.60 cm), leaf number (20.32 leaves plant^{-1}), leaf area (65.98 cm^2), branch number (5.98 branches plant^{-1}), and stem diameter (9.24 mm). significantly outperformed all other treatments and represented the optimal combination for promoting vegetative growth in grapevine cv. Zark transplants under the conditions of this study.

5. The findings of this study indicate that the combined application of 300 g plant^{-1} vermicompost with Bacillus subtilis inoculation is recommended as an effective, sustainable, and environmentally friendly biofertilization strategy for improving vegetative growth and transplant quality of local grapevine cv. Zark.

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