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Studying the Effect of *Bacillus Subtilis* Suspension on Plant Height, Weight and Number of Potato Tubers

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ABSTRACT: ***Background:*** *Bacillus subtilis is one of the microorganisms widely spread in soil. It is characterized by broad activity in producing many metabolites that play an important role against many plant pathogens. In addition, it contributes to increasing the production of plant hormones and facilitating the absorption of many important elements, which reflects positively on the growth and productivity of the plant.* ***Objective:*** *This study aims to investigate the effect of Bacillus subtilis treatment on plant height, weight, and number of potato tubers.* ***Methods:*** *A suspension of Bacillus subtilis was tested over three intermittent periods on plant height and its yield (weight and number of tubers) of the Afamia potato plant compared with the control on two lines of potato without treatment.* ***Results:*** *The relative increase in the average longitudinal growth of the plants in the second line was 49.75% compared to the first line, 29.53%. The average number and weight of tubers were calculated for each replicate of the first-line plants (sprayed with water only), and then the average was calculated for each replicate of the second-line plants (sprayed with bacteria). The average number of tubers in the plants treated with bacteria was 17.56 tubers compared to its counterparts sprayed with water only. In terms of the number of tubers, which amounted to 15 tubers, we obtained a percentage increase of 17.06%. In contrast, the average weight of the tubers was 1618.4 grams compared to its counterpart sprayed with water only, which amounted to 897.6 grams, and thus we obtained a percentage increase of 80.30%.* ***Conclusions:*** *The results showed a positive effect of using Bacillus subtilis on plant height and the number and weight of potato tubers compared to the control. This was explained by the fact that Bacillus Subtilis stimulated the plant's resistance to pathogens and improved plant growth through the production of hormones and facilitating the assimilation and absorption of nutrients.*

KEYWORDS: *Bacillus subtilis; Potato plant; Plant height; Tuber weight; Number of tubers*

INTRODUCTION

Potatoes are considered the third largest food crop in the world after wheat and rice and play a major role in feeding the world [1]. They are widely popular due to their delicious taste and multiple uses [2] and because they contain high nutritional value and are easy to grow even in somewhat different environmental regions [3]. Potato cultivation has a short generation and rapid productivity, produces large quantities on small areas of land, and tolerates climate harshness more than other major crops [4]. Many researchers have studied plant growth promoting factors using Plant Growth Promoting Rhizobacteria (PGPR) with different pathogens on many crops such as tomatoes, cucumbers, peppers, wheat, and others [5]. Growth-enhancing bacteria are microorganisms present in the plant's root area, which work to quantitatively and qualitatively stimulate plant growth and facilitate the plant's absorption of nutrients present in the soil [6]. *Bacillus subtilis (B. subtilis)* bacteria are widely distributed in soil and can produce some secondary metabolites with broad-spectrum antifungal activity against fungi and other plant pathogens [7].

B. Subtilis bacteria have broad activity against pathogens due to their ability to form endospores, which help them resist difficult environmental conditions, in addition to their broad-spectrum antibiotic activity [8]. These bacteria have been used to control the Cnaphalocrocis insect that infects rice plants [9]. *B. Subtilis* XZ18-3 strain was also used as a biocontrol agent on the fungus Rhizoctonia cerealis, which parasitizes wheat roots [10]. The *B. Subtilis* type is distinguished by its ability to produce a large variety of antifungal compounds, the most important of which are: non-ribosomal cyclic lipopeptides [11].

B. Subtilis bacteria show activity against many pathogens, such as Fusarium oxysporum, which showed an inhibition rate of 79%, as well as the fungus Macrophomina phaseolina, by 50% [12]–[15]. Many antibiotics have been isolated that were produced by *B. Subtilis* bacteria, such as Bacteriocins, Lantipeptides, and Polyketides. These antibiotics are characterized by their broad-spectrum activity against fungi [16], as well as many cyclic lipopeptides such as Surfactine, Iturin, and Fengycin. Fengycin plays an important role in enhancing plant growth and resistance against pathogens [17]–[19].

Commercial preparations of strains of *B. Subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a were evaluated for their effectiveness in stimulating tomato plant growth and stimulating systemic resistance against infection with the cucumber mosaic virus and the bacteria Pseudomonas syringae pv. Tomato DC3000 on the herb *Arabidopsis thaliana*. These preparations showed an improvement in plant growth hormones such as auxins, gibberellins, ethylene, salicylic acid, and jasmonic acid [20].

Ryu et al. [21] indicated that a mixture of two *Bacillus* strains can be used as a biostimulant to protect the plant from bacterial and viral diseases on the one side and improve plant growth on the other side. One study also showed that bacteria are used as a biofertilizer and biopesticide, which makes them contribute to enhancing the growth of herbaceous plants such as grains, vegetables, and other important economic plants and increasing their yield [22].

The results of a recent local study indicated the role of the bacteria *B. Subtilis* in improving many growth indicators of pepper plants. The results of the study showed that plants treated with the bacteria *B. Subtilis* FZB27 were significantly superior in many growth indicators such as plant height, root length, wet weight, and dry weight of the shoot and root system compared to plants not treated with bacteria [23]. Another study indicated the effectiveness of the *B. Subtilis* 21-1 strain, BS21-1, in improving plant growth and disease resistance in the conditions of two different types of soil. Treatment with the BS21-1 strain significantly improved plant growth by measuring plant height, leaf width, and seed germination rate [24] . The results of a study showed that treating tomato seeds with bacterial suspensions led to an improvement in the height of the treated tomato plants infected with the cucumber mosaic virus, as stunting rates ranged in the plants treated with the bacteria (5.2%-16.72%) compared to the infected control (23.75% [25].

MATERIALS AND METHODS

Culture Media Used

B. Subtilis bacterium was grown on nutrient agar medium after being isolated and purified from soil. After incubation for 24 hours, a suspension was made from this bacterium according to a McFarland standard. This research was conducted in the field of agricultural land in the university training area at the University of Aleppo from March until July 2022, and in the fungi research laboratory in the College of Science.

Work Stages

Two lines of potato tubers were planted on 3/1/2022, as shown in Figure 1. They belong to the Afamia cultivars variety, obtained from the Seed Multiplication Foundation in Aleppo. The distance between each line and the other is 75 cm. Each line has 10 replicates, each replicate containing 5 plants (so one line contains 50 plants) and the distance between every two tubers is 20 cm (that is, in every 1 meter there are 5 plants).

After the potato tubers germinated and were left to grow and the shoots increased (a month after planting), the first line was sprayed with water only (as a control), while the second line was sprayed with a suspension of *B. Subtilis* bacteria at a concentration of 10^8, as shown in Figure 2, then incubated for a week, considering the appropriate environmental conditions (spray irrigation) for growth. After a week of incubation, spraying was repeated twice with a week's interval between each spray, as in the previous step (the first line with water and the second with *B. Subtilis* at concentration 10^8).

Figure 1. Plant potato lines

Figure 2. Spray bacteria on potato plants

The longitudinal growth of plants was measured as follows: the average length of the plants for the replicates in each line was measured one month after planting (on April 1, 2022) by placing the beginning of the measuring tape at the point where the stem meets the soil and up to the top of the plant. The readings were recorded, and a week after the previous measurement (where the line was sprayed), the first line was with water and the second line was with bacteria. The plant length was re-measured for the replicates in each line and the readings were recorded.

RESULTS AND DISCUSSION

Calculating the Longitudinal Growth of Plants

The results showed that the average longitudinal growth of the replicates of the second line (sprayed with bacteria) was superior to the average longitudinal growth of the replicates of the first line (sprayed with water only), as shown in Table 1, where the relative increase in the average longitudinal growth of the plants in the second line was 49.75% compared to the relative increase in the average growth. The length of the first line was 29.53%, and thus we obtained an increase in growth of 20.22%. This is explained by the fact that the bacteria contributed to increased growth by stimulating long-term systemic resistance of the plant against a wide spectrum of plant pathogens, which improved plant growth [26], increasing the production of plant hormones and facilitating the absorption of phosphate,

Table 1. The differences in the average height of plants in the first and second lines before and after treatment

Repeater number	Average plant height, 1st line, 1 - April - 2022 (cm) Sprayed water only	Average plant height, 1st line, 8 - April - 2022 (cm) Sprayed water only	Average plant height, 2nd line, 1 - April - 2022 (cm) Before sprayed bacteria	Average plant height, 2nd line, 8 - April - 2022 (cm) after sprayed bacteria
Repeater 1	18	23	22	31
Repeater 2	19	25	20	30
Repeater 3	19	24	21	33
Repeater 4	20	25	19	28
Repeater 5	21	26	23	32
Repeater 6	20	27	18	29
Repeater 7	19	24	18	30
Repeater 8	20	26	22	32
Repeater 9	19	24	22	33
Repeater 10	18	20	20	29
Average	19.3	24.4	20.3	30.7

zinc, and potassium and producing iron transporters, or by controlling plant pathogens through the production of antibiotics, HCN, and hydrolytic enzymes such as Chitinase, gluconate [21].

Calculating the Number of Potato Tubers

The average number of tubers was calculated for each replicate of the first-line plants (sprayed with water only), and then the average was calculated for each replicate of the second-line plants (sprayed with bacteria), presented in Table 2.

Table 2. The average number of tubers for the first line sprayed with water and the second line sprayed with bacteria

Repeater number	Average number of tubers, 1st line, water only	Average number of tubers, 2nd line, bacteria
Repeater 1	13.4	17
Repeater 2	14.4	16.8
Repeater 3	15.8	18
Repeater 4	14.6	17.4
Repeater 5	15.4	18.2
Repeater 6	15.8	18.6
Repeater 7	16.4	17.6
Repeater 8	15.6	16.8
Repeater 9	14.6	17.2
Repeater 10	14	18
Average	15	17.56

The study showed that *B. Subtilis* extracts lead to improved plant growth and increased productivity. As shown in Table 1, the statistical analysis showed that the treatments in the second line sprayed with bacteria were superior to the treatments in the first line sprayed with water only. The average number of tubers in the plants treated with bacteria was 17.56, compared to its counterpart sprayed with water only in terms of the number of tubers, which amounted to 15. Thus, we obtained a percentage increase of 17.06%, which indicates that *B. Subtilis* has a positive effect in improving the growth and productivity of the potato plant in terms of the number of tubers.

Calculating the Weights of Potato Tubers

Similarly, the average tuber weight was calculated for each replicate of the first-line plants (sprayed with water only), as well as the average for each replicate of the second-line plants (sprayed with bacteria), as shown in Table 3.

Table 3. The average weight of tubers for the first line sprayed with water only and the second line sprayed with bacteria

Repeater number	**Average weight of tubers, 1st line, sprayed with water only (grams)**	**Average weight of tubers, 2nd line, sprayed with bacteria (grams)**
Repeater 1	968	1640
Repeater 2	920	1632
Repeater 3	984	1704
Repeater 4	760	1576
Repeater 5	928	1664
Repeater 6	952	1712
Repeater 7	1000	1560
Repeater 8	808	1472
Repeater 9	880	1536
Repeater 10	776	1688
Average	897.6	1618.4

From Table 3, the statistical analysis showed that the treatments in the second line sprayed with bacteria were superior to the treatments in the first line sprayed with water only in terms of the weight of potato tubers. The average weight of the tubers was 1618.4 grams compared to its counterpart sprayed with water only, which amounted to 897.6 grams. Thus, we obtained a percentage increase, by 80.30%, which indicates that *B. Subtilis* improves the growth and productivity of potato plants in terms of tuber weight. Therefore, the bacteria helped increase the yield of the potato crop. This is explained by the fact that the bacteria helped the plant to grow better by increasing the production of plant hormones and facilitating the absorption of some nutrients from the soil. The results of this study were consistent with a study that showed *B. Subtilis* bacteria produce volatile compounds that prevent the growth of pathogens on the plant, which leads to better crops [27]. It also agreed with a local study conducted on pepper plants in terms of the positive effect of *B. Subtilis* bacteria on plant growth, while it differed from the concentration used is 1010 in the local study, while in our study 108, as well as in the method of applying the treatment, in local study by soaking the seeds with bacterial suspension, while in our study it was sprayed on the vegetative parts of the plant after germination [28].

A one-way analysis of variance was conducted between the treatments in the first line (spraying with water only) and the second (spraying with bacteria at a concentration of 108 in three batches, the first on 1 April 2022, the second on 8 April 2022, and the third on 15 August 2022, during cultivation), so the P value was - value=0.00 this confirms the presence of significant differences in the number of tubers between the studied treatments, as shown in Table 4.

Table 4. The differences between the means for the number of tubers between treatments 1 and 2

Transaction status	**Transaction number**	**Mean ± standard deviation**
Spray with water only	1	15±0.94
Spray with bacteria only	2	17±0.62

It is clear from Table 4 that there are significant differences in the number of tubers between the first and second lines. The second line (sprayed with *B. Subtilis*), in which the number of tubers reached 17 tubers, is significantly superior to the first line (sprayed with water only), in which the average number of tubers reached 15 tubers.

We conducted an analysis of variance for the same previous coefficients regarding the weight of potato tubers, and they were P-value=0.00 This also confirms the presence of significant differences in tuber weights between the studied treatments, as shown in Table 5.

Table 5. The differences between the averages in tuber weights in grams between treatments 1 and 2

Transaction status	Transaction number	Mean ± standard deviation
Spray with water only	1	897.6±21.95
Spray with bacteria only	2	1618.4±19.91

It is clear from Table 5 that there are significant differences in the average weight of tubers between the first and second lines. The second line (sprayed with the bacteria *B. Subtilis*), where the average weight of tubers reached 1618 grams, is significantly superior to the first line (sprayed with water only), which had an average weight of tubers 898 gr, as shown in Figure 3.

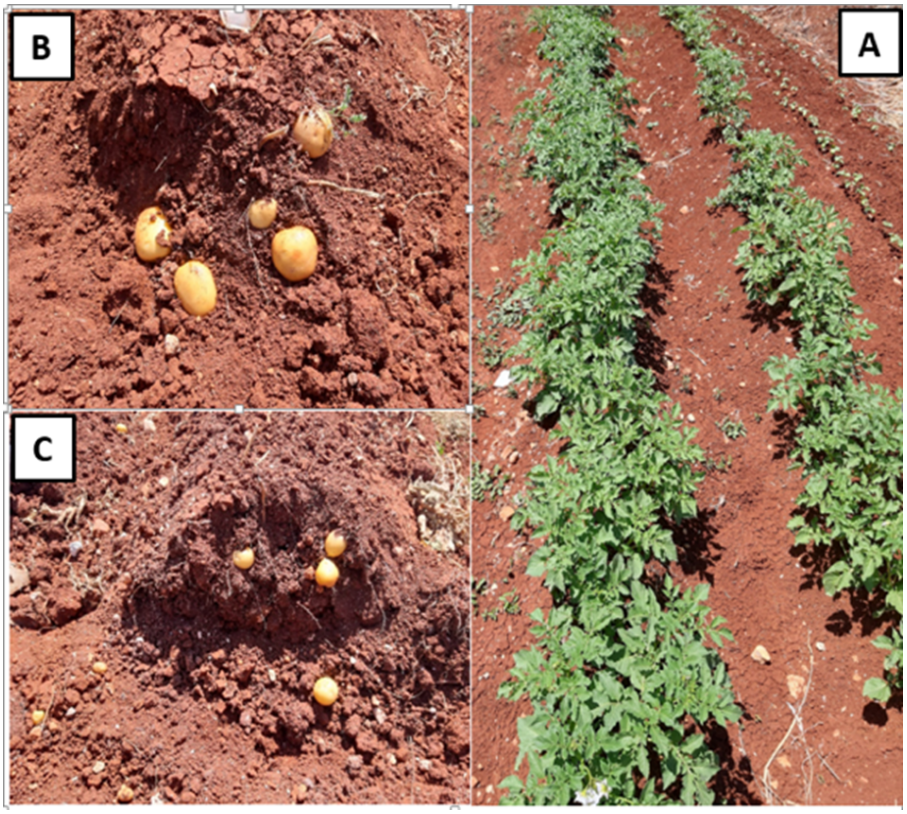

Figure 3. A: The difference in shoots between Line No. 1 (on the right) sprayed with water only, and Line No. 2 (on the left) sprayed with bacteria. B, C: The difference in the number and size of tubers between Line 1 (C) sprayed with water only, and Line 2 (B) sprayed with bacteria

CONCLUSION

The bacteria *B. Subtilis* enhanced the growth of the plant's shoots and protected it from infection by pathogens. Treating potato plants with *B. Subtilis* bacteria at concentration 108 contributed to enhancing plant growth in terms of increasing the number of tubers, and the increase rate was 17.06%. Treating potato plants with *B. Subtilis* bacteria at concentration 108 also contributed to enhancing plant growth in terms of increasing the weight of tubers, and the percentage of increase was 79.85%.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Anwar Mohammed Khalil: Formal analysis, Visualization, Writing - review and Editing. Abdel Moneim Ahmed: Supervising the laboratory side of the experiments, such as cultivating and preparing the fungal spore suspension. Marwan Abdo Hassan: Supervising the field side of the experiment and the method of preparing the suspensions and spraying them on the plant.

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DATA AVAILABILITY STATEMENT

None.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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