The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)
ISSN: 2791-8033

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2022

Volume 5, Pages 12-27

ICMeHeLS 2022: International Conference on Medical, Health and Life Sciences

Exploring the Use of Bacillus Subtilis to Improve the Growth of Phaseolus Vulgaris under Saline Conditions

Shamma ALGHAFRI
Zayed University

Abstract: Soil salinity is a problem that affects many countries, and one of them is the United Arab Emirates. A microorganism that has been known for its salinity resistance mechanisms could be a factor in improving and enhancing agricultural problems. The aim of this study is to explore the use of Bacillus Subtilis in agricultural practices and how it can affect growth under saline conditions and guide future studies in this field with the goal of giving better implications and providing more information. Bacillus Subtilis was introduced to a plant that cannot tolerate high salinity levels, Phaseolus Vulgaris. Three different methods were used in conducting this study: (1) germination of seeds in bacterial broth, (2) inoculation of seeds with bacterial broth, and (3) spreading of bacterial broth on soil samples. This study confirmed that using Bacillus Subtilis with Phaseolus Vulgaris indeed increases its resistance in saline conditions. Moreover, using this bacterium could be the solution to agricultural problems due to high salinity levels if they were engineered and modified to enhance their resistance tolerance. The recommendations to this study would be to implement it in a controlled field and explore the usage of Bacillus Subtilis with different plants and soils as well as to check how other factors, such as temperature, affect the growth of plants in saline conditions while Bacillus Subtilis is present in that environment. Another recommendation would be collecting data with different methods and using DNA sequencing to check how Bacillus Subtilis affect soils and the microbes that exist in those soils.

Keywords: Soil Salinity, Bacillus Subtilis, Phaseolus Vulgaris, Agriculture.

Introduction

Agriculture is one of the oldest professions in the human history, and it is the key to obtaining food. There are many subsidiary branches of agriculture like fish farming, bee keeping, animal husbandry, etc. Agriculture in UAE was mainly dependent on fishing during the period between 1970 and 1990s, contributing 4% in total GDP of USE economy. Lack of proper rain, salinity, high temperature, and sandstorms make things very difficult for agriculture in the UAE and limits the agricultural area. The main farming areas were Diganda in Ras al-khaimah, Falaj al Mulla in umm al Qawain, wadi adhDhayad in Sharjah; total of only 70,000 hectares land was available for agriculture. The severe problem of salinity in the UAE should be tackled to increase agricultural food yield. According to Machado &Serralheiro, (2017) It was estimated that about 20% of irrigated land, producing one-third of the world's food, is salt-affected. In the European Union around 1 hectare are affected due to high salinity levels, this is a major cause of desertification. In Spain, 3% of the 3.5 million hectares of irrigated land is severely affected, markedly reducing its agricultural potential, while another 15% is under serious risk (Machado & Serralheiro, 2017). Salinity both affects soil and plant growth, affecting osmotic pressure and to interference with plant nutrition. High salinity level in soil reduces the ability of plants to retain water, often referred as water-deficit effect. It causes metabolic changes in plant due to ions toxicity and lack of nutrition balance. To counter the problem like salinity of the soil, scientists are working on different methods such as genetically modified plants, genetic engineering, biotic agents like viruses, bacteria, fungi, algae. The biotic agents induce biotic stress in the host by interfering in their metabolism, but sometimes these biotic agents interact to host symbiotically. These microbes are beneficial to the plants such as lichen, mycorrhizae, bacillus subtills. These organisms also get food, shelter, nutrients in return. Bacillus subtilis helps plant to grow in harsh

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

saline condition by restricting the uptake of sodium ion by decreasing the passive flow of sodium ion into the stele, since these Bacillus subtilis covered the maximum proportion of roots in specifically PGPR-inoculated plants. Another way Bacillus Subtilis protects plants against salt is by lowering ROS levels in plants. This protects plants from oxidative stress such as protein oxidation, enzyme inhibition, and poor chlorophyll content. According to Ferria et al (2018), the ability of Rhodopseudomonas palustris (strains TN114 and PP803) EPS to adsorb Na cations from aqueous solution is due to an 18 kDa polysaccharide consisting primarily of galacturonic acid (Ferria et al, 2018). This facilitates the potassium uptake in the plants prevents from ions poisoning. The aim of this study is to explore the use of Bacillus Subtilis in agricultural practices and how it can affect growth under saline conditions and guide future studies in this field with the goal of giving better implications and providing more information.

Literature Review

Soil Salinity & Agricultural Activities

Soil salinity is a measurement that consists of the concentration of all soluble salts in soil water. Some of the soluble salts are cations such as sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}); the anions are chloride (Cl^-), carbonate ($CO_3{}^{2-}$), sulfate ($SO_4{}^{2-}$), bicarbonate ($HCO_3{}^-$), and nitrate ($NO_3{}^-$). Soil salinity is a problem that is being faced globally, high salinity levels in soil result in land degradation and inhibit plant growth which creates a reduction in crop yield. Hyper saline soils can accommodate boron (B), strontium (Sr), lithium (Li), manganese (Mn), and fluorine (F); those are harmful to certain plants and can reduce their growth and yield rate. This is a serious issue that is being faced in various areas of the globe; it results in agricultural problems and could negatively affect food security around the world. Soil salinity is linked to a variety of difficulties in plants, including ion toxicity, oxidative stress, and osmotic stress (Shahid, S et al., 2018; Yan, K et al., 2013). Increased soil salinity in agricultural activities results in reducing osmotic potential which causes a reduction in turgor in plants and water stress. Salinity sources could be natural, anthropogenic, or both together. When it comes to anthropogenic sources of salinity those can be irrigation with saline water and poor soil management, or soil pollution due to the excessive use of fertilizers (Lopez-Alvarez et al., 2021).

Soil & Soil Salinity in the United Arab Emirates

The soil in the United Arab Emirates is known to be poorly developed, shallow, and rich in gypsum, lime, and salts (Rao et al, 2009). A soil map of the United Arab Emirates has been completed by the Environment Agency – Abu Dhabi; the soil map of Abu Dhabi, which was published in 2009, covers around 84% of the United Arab Emirates. In 2012, EAD published a map for the Northern Emirates, and it made up 11% of the UAE (EAD, 2009; EAD, 2012). Another soil map published in 2005 by Dubai Municipality showed that Dubai makes up 5% of the UAE's soil (Dubai Municipality, 2005).

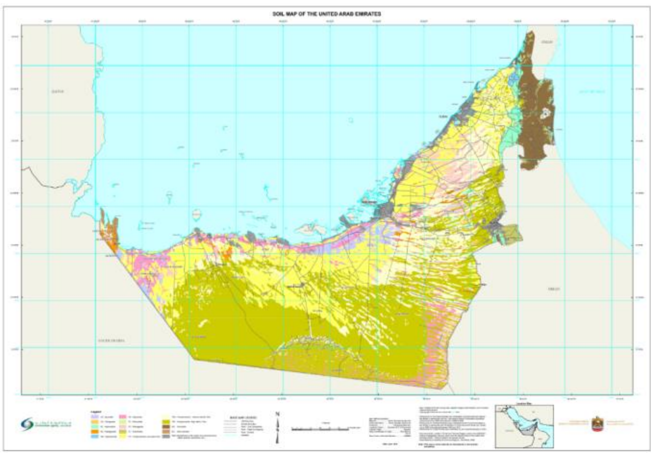

Figure 1. Soil Map of the United Arab Emirates. *Adapted from "Unifying regional soil maps at different scales to generate a national soil map for the United Arab Emirates applying digital soil mapping techniques" by Abdelfattah, M. A., & Pain, C., 2012, Journal of Maps, 8(4), 392-405.*

Ecosystems in the United Arab Emirates include coasts, islands, mountainous areas, sand deserts, and gravel plains (Boer, 1999). Soil salinity is becoming a major threat to agricultural activities; a study showed that around 11% of farms in Al Ain are classified as “non-saline”, and 89% are affected by high salinity levels. Figure 1., Shows different soil types spread around the United Arab Emirates and they are a total of 15 types; 75% Torripsamments, 5.4% Haplosalids, 0.9% Torriorthents, 0.2% Haplocambids, 0.2% Calcigypsids, 0.1% Petrocalcids, 3.3% of units such as mountains, rock outcrops, miscellaneous units (Abdelfattah, M., 2012).

Saline soils are divided into three categories: (1) saline soils, (2) saline gypsifeours, and (3) saline anhydritic soils. In the United Arab Emirates, these three categories were distributed as sabkhas (a supratidal mudflat with saline minerals accumulating due to the arid climate); sabkhas evaporate, resulting in salt accumulation and reaching agricultural farms where brackish water is used for irrigation, and sabkhas have the highest levels of salinity. The classification of Sabkhas are Salids which are classified further at the level of Aquisalids and Haplosalids (Shahid, S., & Abdelfattah, M. A., 2008).

Plant Growth-Promoting Rhizobacteria

Although bacteria were discovered in late 1600s, their utilization to stimulate the plant and its growth has been exploited time to time. Plant promoting rhizobacteria also abbreviated as (PGPR) come under rhizosphere class of bacteria. They inhibit the soil ecosystem, and they are often found with plant roots, and they are available in high concentrations due to the rhizosphere, which has a high amount of nutrients such as amino acids, sugar, and organic acid molecules; they are crucial elements for bacterial growth and metabolism. Gray and Smith (2005) defined it as follows; The genus is as follow- agro bacterium, Azobacter, Aspergillum, Caulobacter, chromobacterium, Erwinia, micrococcus, pseudomonas, and Serratia belongs to ePGPR (Gray & Smith, 2005). Moreover, Frankia endophytes come under iPGPR they enhance plants development and its growth by using different mechanisms, that are phosphate solubilization siderophore production, fixing the atmospheric nitrogen into the soil and Rhizosphere engineering, quorum sensing signal interference, production of 1-aminocyclopropane-1-carbocylate deaminase, etc. as per the author Hasheem et al (2019) Bacillus subtilis exhibits both a direct and indirect biocontrol mechanism to suppress disease caused by pathogens. The direct mechanism includes the synthesis of many secondary metabolites, hormones, cell-wall-degrading enzymes, and antioxidants that assist the plant in its defense against pathogen attack (Hasheem et al, 2019). The indirect mechanism comprises plant growth promotion and the induction of acquired systemic resistance. Bacillus subtilis can also solubilize soil P, enhance nitrogen fixation, and produce siderophores that promote its growth and suppresses the growth of pathogens (Saxena A et al., 2005). When exposed to constant stressors, rhizobacteria are more likely to tolerate or adapt to those conditions. This results in better plant growth, and it acts as a growth promoter under stressors. When exposed to stressors they produce osmoprotectants such as $K+$, glutamate, proline, ectoine, etc (Grover et al., 2010). Hashem et al (2019) stated that Pseudomonas and Bacillus are predominant genera of PGPR.

Salinity Tolerance Mechanisms of Bacillus Subtilis Bacteria

Salinity in agriculture is a major problem. This causes loss in crop production, low yield of crop, poor harvest etc. Saline conditions negatively affect the dry weight of inoculated and non- inoculated plants. For tackling this situation scientist have been working on different solution this causes reduction in 85 to 95 % in shoot and root. Due to the morphology, biochemistry, and physiology of bacteria bacillus subtilis, it helps in improving the tolerance of plants from soil salinity. Bacillus subtilis restrict the uptake of Na ion in the roots of plants by cutting the flow of sodium passively into the stele, this is maintained by covering the root zones by PGPR-inoculated plants. Further studies also connotate, according to the author Ferria et al, (2018) at the highest salinity concentration (200mM) there was an increase in the concentration of proline in treatment without B. subtilis as compared with the presence of Bacillus Subtilis. Its accumulation in plants provides protection against salinity and drought stress. It can be associated with content of water in leaves since there is a strong correlation between water potential in leaves and the concentration of proline (Ferria et al, 2018).

Halophilic Bacteria

High tolerance of plants towards salinity in presence of *bacillus subtills* allowed the rediscovery of this class of saline resistant bacteria commonly known as Halophiles. They need high concentrations of salt to grow. They are classified into the domain Archaea. They can be characterized under different genus and different species.

According to Grover, M., and Sandhya, V., (2010) some of the examples of halophiles are - Halobacteriaceae, Halobacterium, Halococcus. some genus of diatoms such as Nitzschia, lovenula, comes under halophiles as well." Some basidiomycete fungus, genus name- Wollemi Ichthyophage. Chromohalocter, Beijerinckii, Tetragenococcus halophilus are some of the examples. Halophiles can withstand up to high saline concentration places like deep sea lagoons etc. This function must be taken into consideration for the future purpose of solving the saline soil problems. A lot can be learned such as the mechanism of saline tolerate, their interaction with humas, uses from these halophiles in our day-to-day life. Halophiles can help in solving the vast problem of UAE agriculture related problems. Taking account of these things we can truly help humankind and pays a great deed to the environment, but we have also investigated all aspects of humans and nature.

Risk Assessment (Human Health Risk, Environmental Risk)

In past few decades humans have been exploiting the nature, by using different means such as extensive use of fertiliser, pesticide, chemicals, in the field. This leads to serious consequences of human health as well as environmental risk. For overcoming these issue's, we must shift our focus to natural resources provided to us by planet earth. According to the Food and Drug Administration (FDA); Bacillus subtilis is classified as GRAS (Generally Regarded as Safe), therefore, it is not a pathogen (Martinez, 2013). It blocks the accumulation of sodium ion by covering the plants roots, this helps in overcoming the serious issue of nutrients toxicity in plants. In agriculture it is widely used as feed additive and animal husbandry since it inflates the digestion of animal fibre and has potential of maintaining intestinal health of ruminants. Recent studies show its self-healing and viscoelastic behaviour. This evidence clearly suggest that Bacillus Subtilis has positive impact on the human health as well as overcoming the environmental risk. This also opens a path of biotic agents in day-to-day usage. According to Hashem et al., (2019) We use the two well established mixture toxicity concepts (Concentration Addition (CA) and Independent Action (IA)) for providing a tiered outline for environmental hazard and risk evaluations of mixes, with an emphasis on general industrial chemicals and the assumption that the "base set" of data (EC50s for algae, crustaceans, and fish) is available (Hashem et al., 2019). Therefore, we can conclude here that Bacillus Subtilis are not harmful to humans, nor are they a risk to the environment.

Method

Bacterial Suspension for Bacillus Subtilis Sample

Luria Bertani broth was prepared, and 20 ml was added to four sterile 50 ml falcon tubes. The metal rod was heated until sterile and used to take bacterial colonies from the prepared spread plates, and then placed in three falcon tubes with the LB broth, while leaving one tube without the B. Sub colony as a control measure. The tubes were sampled and inoculated, and then placed in the shaker for 18 hours at 25°C. Once the cycle in the shaker is finished; checked for contamination in the control tube, and the LB broth was still clear which conclude that the sample was not contaminated. The samples were placed in the centrifuge at 45000 RPM for 4 minutes; the bacterial colonies were at the base while the LB broth was on the top. The media on the top of the sample was poured out and 10 mM $MgCl_2$ has been diluted in 90 mM distilled water. The $MgCl_2$ has been prepared using the following method:

0.95g of MgCl2 was mixed in 100 ml deionized water
1 Molar (0.95 g) × 100 ml = 100 mM MgCl2

The samples have been placed in the centrifuge for a second time and the same steps were repeated for 3 times total.

Methods Used to Introduce Bacillus Subtilis to Phaseolus Vulgaris

Seed germination in B. Subtilis bacterial suspension: Seeds were left to germinate in distilled water unit they start exposing and the outer shell cracks open, once they reach this stage of germination, they were placed in petri dishes and submerged with the B. sub bacterial suspension solution and were kept for a week, and observations were done regularly.

Seed Inoculation with B. Subtilis bacterial suspension: Seeds were left to germinate until the outer shell cracks and expose the roots; using a sterile syringe the roots were gently poked and then submerged with the B. sub bacterial suspension solution and left for a week with regular observations.

Spreading B. subtilis bacterial suspension on soil: The B. sub bacterial suspension was spread on the agricultural soil sample.

Collecting Data on Plant Growth and Analysis

Phenospex trait finder was used to collect data on plant growth in the span of 3 weeks after the germination and potting the seeds in saline soil of different concentrations. From the data collected all the analysis done in the discussion focused on one morphological parameter which is the plant height, and one spectral index which is normalized digital vegetation index "NDVI". The data from the trait finder was taken and organized in tables and bar graphs.

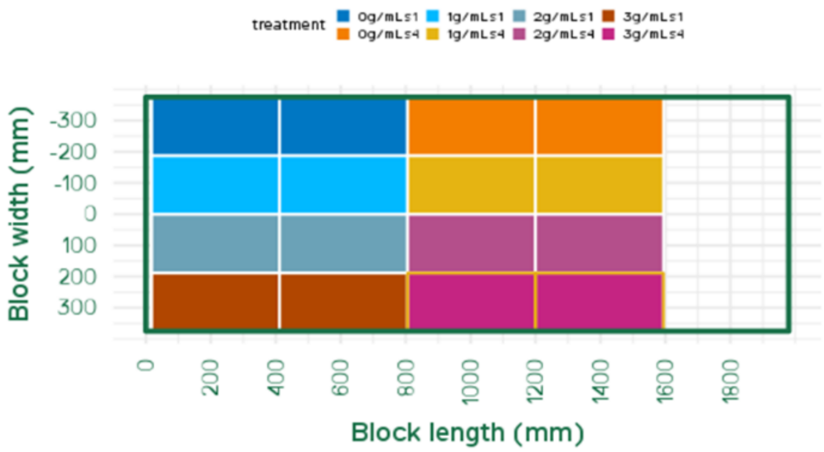

Figure 2. Pots placement on the phenospex based on their % salinity
(Note: S1, S2, S3, and S4 refers to the group number)

Table 1. Shoot height in week 1 of phaseolus vulgaris in different growth conditions with bacillus subtilis

	Treatment	T1 Height (cm)	T2 Height (cm)	Average
Group 1 (Seeds germinated in Bacillus subtilis)	0g/mL	18.97	9.23	14.10
	1g/mL	18.21	17.29	17.75
	2g/mL	-	2.96	2.96
	3g/mL	0.46	15.03	7.74
Group 2 (Seeds inoculated with Bacillus subtilis)	0g/mL	20.58	17.24	18.91
	1g/mL	18.20	17.61	17.91
	2g/mL	17.22	9.23	13.22
	3g/mL	12.84	6.28	9.56
Group 3 (Bacillus Subtilis Spread on Soil)	0g/mL	20.75	21.25	21.00
	1g/mL	17.94	16.64	17.29
	2g/mL	15.17	15.33	15.25
	3g/mL	16.88	17.51	17.20
Group 4 (No Bacillus Subtilis)	0g/mL	18.51	14.75	16.63
	1g/mL	11.53	11.50	11.52
	2g/mL	3.37	9.69	6.53
	3g/mL	6.53	13.36	9.94

Results and Discussion

Shoot Height Growth of Phaseolus Vulgaris in the time span of three weeks after germination

Shoot Height Growth Data Analysis

The shoot height of Phaseolus Vulgaris was taken once a week using the phenospex in the span of 3 weeks. Group 1 refers to the samples which were left to germinate in Bacillus subtilis for a week, group 2 refers to the samples which were germinated and once the roots were visible inoculated in Bacillus subtilis, group 3 refers to the third method used; spreading Bacillus subtilis on the soil directly. The experiment conducted had two trials and the data was collected for each trial, therefore, the data used on graphs was averaged from trial 1 and trial 2.

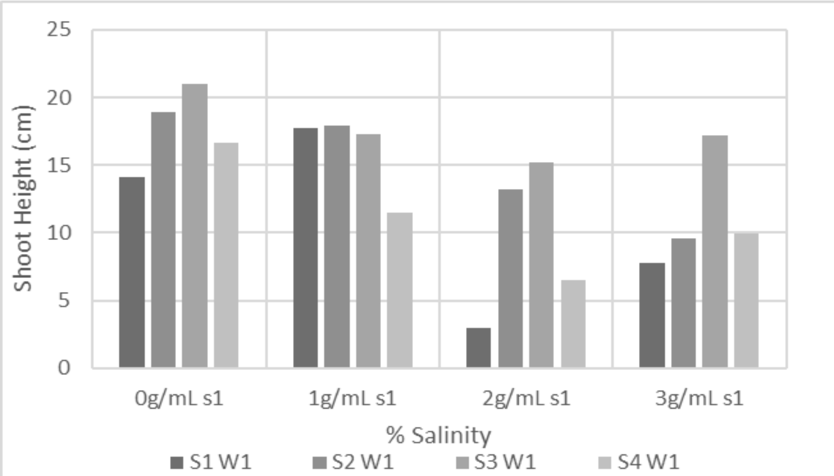

Figure 3. Shoot height growth week 1

Table 1 shows the data obtained from the phenospex for trial 1 and trial 2 in week 1. The data includes all the four groups, and *figure (2)* is based on the averages for trial 1 and 2. *Figure (2)* compares different groups in different salinity levels; group 3 had the highest shoot average (21 cm) in 0% salinity, and (17.2 cm) at 3% salinity. While the sample from group 1 had the lowest shoot average (3 cm) at 2% salinity.

Table 2. Shoot height in week 2 of phaseolus vulgaris in different growth conditions with bacillus subtilis

	Treatment	T1 Height (cm)	T2 Height (cm)	Average
Group 1 (Seeds germinated in Bacillus subtilis)	0g/mL	26.31	18.78	22.54
	1g/mL	17.21	17.95	17.58
	2g/mL	-	19.52	19.52
	3g/mL	15.56	3.35	9.46
Group 2 (Seeds inoculated with Bacillus subtilis)	0g/mL	17.79	25.38	21.59
	1g/mL	18.25	20.94	19.59
	2g/mL	16.04	8.43	12.24
	3g/mL	14.81	5.75	10.28
Group 3 (Bacillus Subtilis Spread on Soil)	0g/mL	23.62	24.00	23.81
	1g/mL	17.76	16.18	16.97
	2g/mL	14.52	14.38	14.45
	3g/mL	15.20	10.14	12.67
Group 4 (No Bacillus Subtilis)	0g/mL	19.41	14.55	16.98
	1g/mL	11.66	11.68	11.67
	2g/mL	3.19	9.95	6.57
	3g/mL	6.38	12.75	9.57

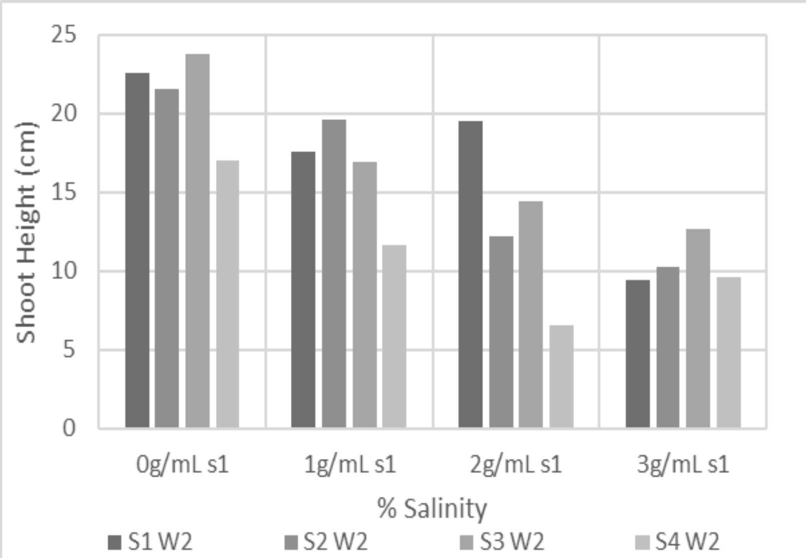

Figure 4. Shoot height growth week 2

Table 2 shows the data obtained from the phenospex for trial 1 and trial 2 in week 2. The data includes all the four groups, and *figure (4)* is based on the averages for trial 1 and 2. In week 2 we can notice a drastic change in figure (4); there was a sharp surge in the shoot height average for group 1 at 2% salinity (19.5 cm), and at 1% salinity (22.5 cm).

Table 3. Shoot height in week 3 of phaseolus vulgaris in different growth conditions with bacillus subtilis

	Treatment	T1 Height (cm)	T2 Height (cm)	Average
Group 1 (Seeds germinated in Bacillus subtilis)	0g/mL	28.24	22.37	25.30
	1g/mL	15.86	18.19	17.03
	2g/mL	-	19.59	19.59
	3g/mL	12.69	-	12.69
Group 2 (Seeds inoculated with Bacillus subtilis)	0g/mL	26.27	21.07	23.67
	1g/mL	18.41	21.01	19.71
	2g/mL	14.88	8.86	11.87
	3g/mL	8.45	5.33	6.89
Group 3 (Bacillus Subtilis Spread on Soil)	0g/mL	18.86	26.59	22.72
	1g/mL	17.21	14.60	15.91
	2g/mL	15.18	15.95	15.56
	3g/mL	2.78	6.84	4.81
Group 4 (No Bacillus Subtilis)	0g/mL	21.79	13.46	17.62
	1g/mL	10.81	11.77	11.29
	2g/mL	2.15	9.95	6.05
	3g/mL	3.07	7.80	5.43

Table 3 shows the data obtained from the phenospex for trial 1 and trial 2 in week 3. The data includes all the four groups, and *figure (2)* is based on the averages for trial 1 and 2. In week 3 we can observe that the data obtained from group 2 had the highest averages in all salinity levels except for 1% salinity level. The average for 0% salinity level has reached (25.3 cm), and in 3% salinity the plant was still alive and there was a minimum difference in comparison to week 2.

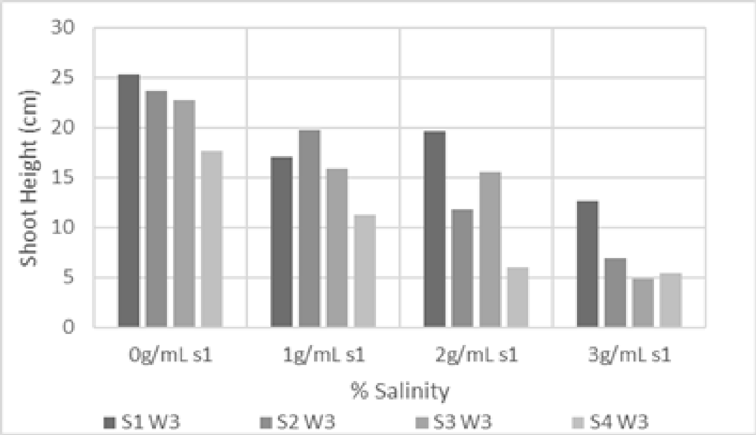

Figure 5. Shoot height growth week 3

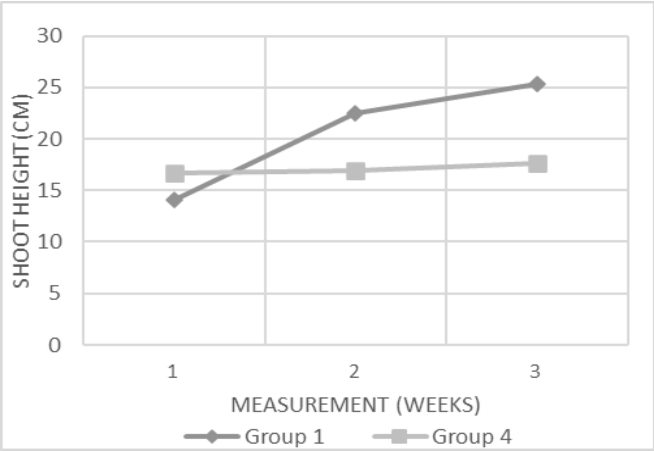

Figure 6. Group 1 & 4 shoot height growth measurement of 3 weeks with 0% salinity.

Figure (6) illustrates the differences between group 1 and group 4 in 0% salinity in a measurement of 3 weeks. When looking into the data and comparing group 1 & 4 in 0% salinity we can notice normal growth for group 4, while group 1 (germinated in B. Sub) we can notice a slightly more elevated growth with more leaf count and flower yield. In image 7, which was taken on week 3, we can notice the difference between group 1 and group 4. However, when looking into this set of data we need to compare it to another set to be able to tell how the bacteria are working and if they are effective in higher salinity levels.

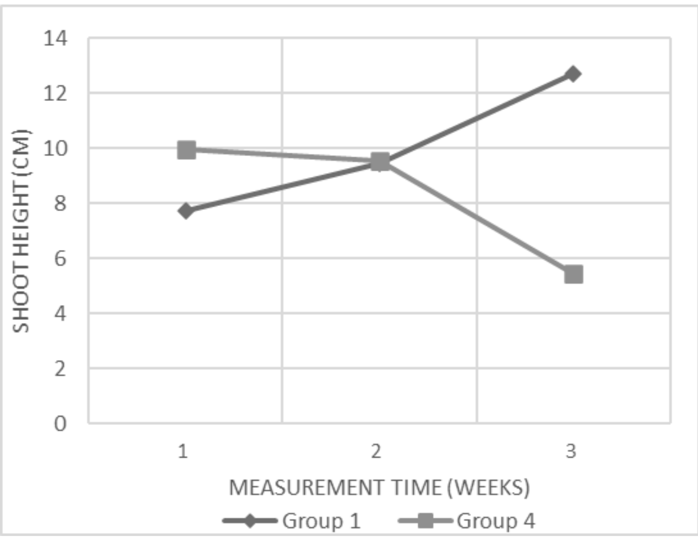

Figure 7. Group 1 & 4 shoot height growth measurement of 3 weeks with 3% salinity.

Figure (7) illustrates the differences between group 1 and group 4 in 3% salinity in a measurement of 3 weeks. When looking into the data from group 1 & 4 in 3% salinity, we can notice a varying difference between the two groups. Unlike the data from 0% salinity, here we can notice an elevation in the data for group 1 which has the bacillus subtilis compared to group 4 which has no b. sub in it; according to Hashem, Tabassum & AbdAlla (2019) the rhizosphere has most nutrients of plants, and bacteria accumulate there for the nutrients, in exchange the bacteria helps the plant by providing protection from abiotic stress due to the bacillus subtilis bio-film which stays on the roots for the long term and protect and stimulates growth.

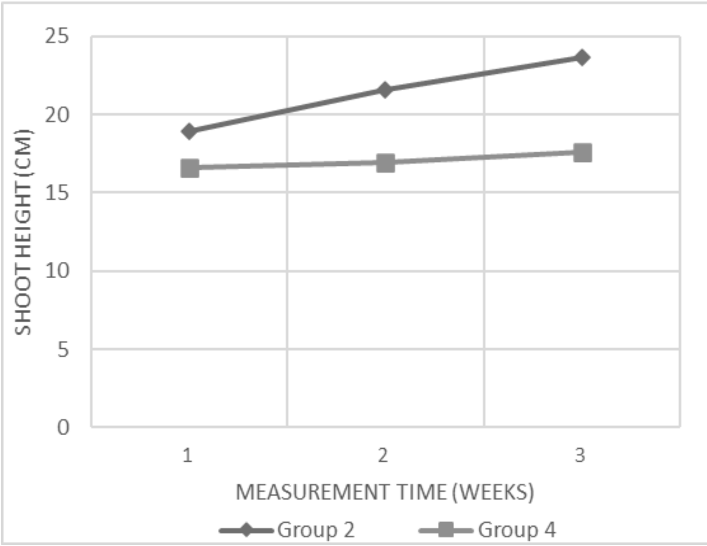

Figure 8. Group 2 & 4 Shoot height growth measurement of 3 weeks with 0% salinity.

The experiment conducted used 3 different methods to introduce Bacillus subtilis into Phaseolus Vulgaris in saline conditions; the first experiment was germination of seeds in B. sub suspension sample, second experiment was inoculation of roots with B. sub suspension sample, and the third one was spreading the B. sub sample on the soil directly. *Figure (8)* illustrates the differences between group 2 and group 4 in 0% salinity in a measurement of 3 weeks. When looking into the data and comparing group 2 & 4 in 0% salinity we can notice normal growth for group 4, and group 2 had the same growth level as group 1 in 0% salinity. However, group 1 had more growth compared to group 2.

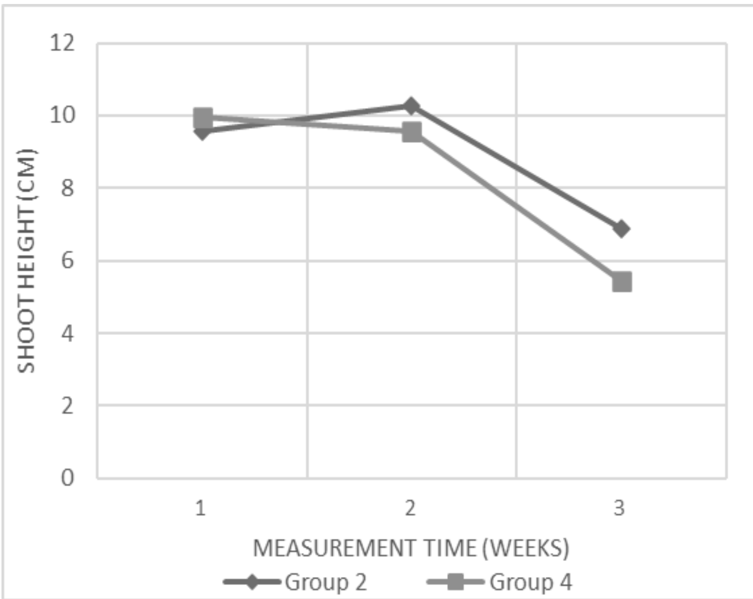

Figure 9. Group 2 & 4 shoot height growth measurement of 3 weeks with 3% salinity.

In contrast when we look at the data in *Figure (9)* for group 2 and 4 in 3% salinity, we can notice a sharp decline in the trendline. Group 2 was inoculated, and the roots were poked to allow B. sub to have an entrance to the plant. However, as mentioned above in the section *4.2* the roots showed signs of injury and the sharp increasing in salinity could have added more stress.

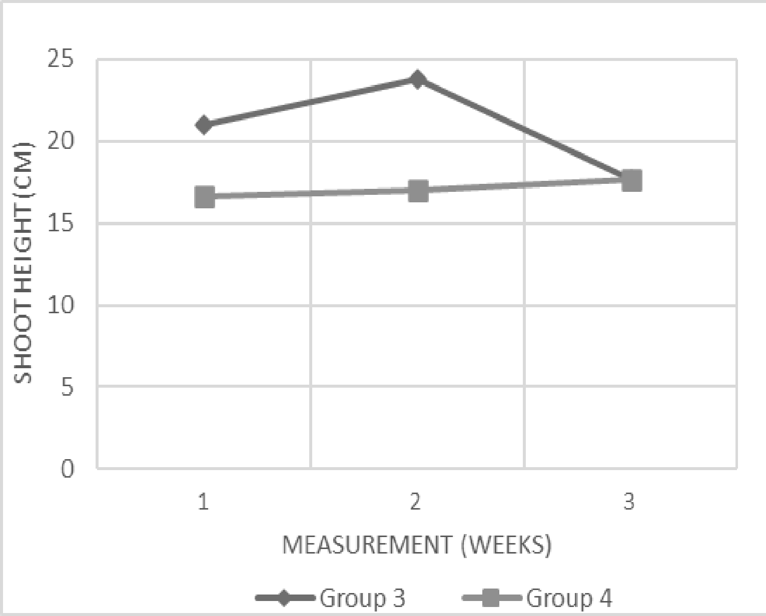

Figure 10. Group 3 & 4 Shoot height growth measurement of 3 weeks with 0% salinity.

Figure (10) illustrates the differences between group 3 and group 4 in 0% salinity in a measurement of 3 weeks. When looking into the data and comparing group 3 & 4 in 0% salinity we can notice normal growth for group 4, and during the first two weeks group 3 was growing at a higher rate and height average, however, in week 3 the height average was the same as group 4 in week 3.

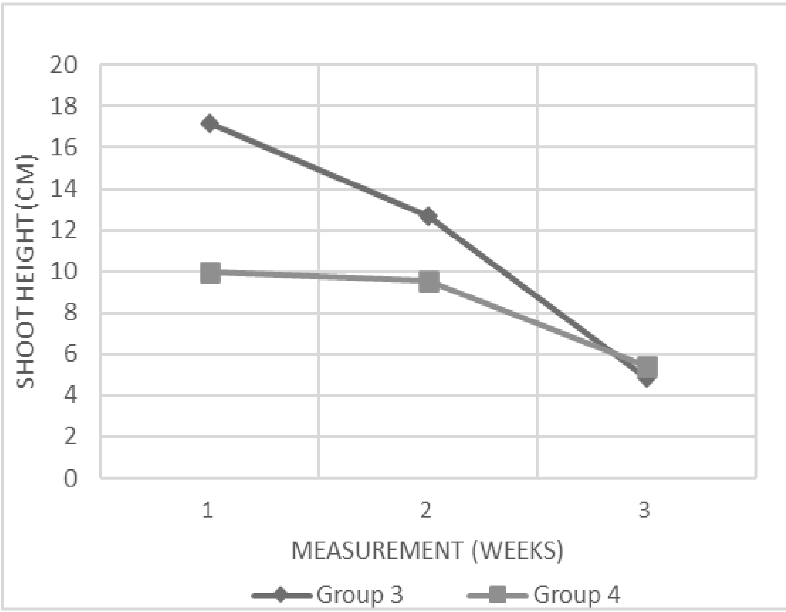

Figure 11. Group 3 & 4 shoot height growth measurement of 3 weeks with 3% salinity.

As shown in Figure (11); group 3 and 4 in 3% salinity data showed a sharp decline in the trendline. Both groups reached a similar average in height in week 3 and both groups started to wilt.

Normalized Difference Vegetation Index (NDVI) Data Analysis

The NDVI index ranges from -1.0 to 1.0. Values below 0.1 indicate bare soil, 0.1 – 0.5 are sparse vegetation, and dense vegetation ranges from 0.6 and above. NDVI is directly related to photosynthetic activity of the vegetation and indirectly related to crop yield and the quality of the environment as habitat for various factors like pests and diseases.

Table 4. NDVI in week 1 of phaseolus vulgaris in different growth conditions with bacillus subtilis

	Treatment	T1 NDVI	T2 NDVI	Average
Group 1 (Seeds germinated in Bacillus subtilis)	0g/mL	0.77	0.76	0.77
	1g/mL	0.75	0.76	0.75
	2g/mL	-	0.42	0.42
	3g/mL	0.51	0.74	0.63
Group 2 (Seeds inoculated with Bacillus subtilis)	0g/mL	0.75	0.79	0.77
	1g/mL	0.80	0.78	0.79
	2g/mL	0.62	0.61	0.62
	3g/mL	0.72	0.63	0.67
Group 3 (Bacillus Subtilis Spread on Soil)	0g/mL	0.71	0.72	0.72
	1g/mL	0.77	0.78	0.77
	2g/mL	0.79	0.78	0.78
	3g/mL	0.61	0.75	0.68
Group 4 (No Bacillus Subtilis)	0g/mL	0.73	0.68	0.71
	1g/mL	0.76	0.74	0.75
	2g/mL	0.67	0.74	0.71
	3g/mL	0.72	0.53	0.63

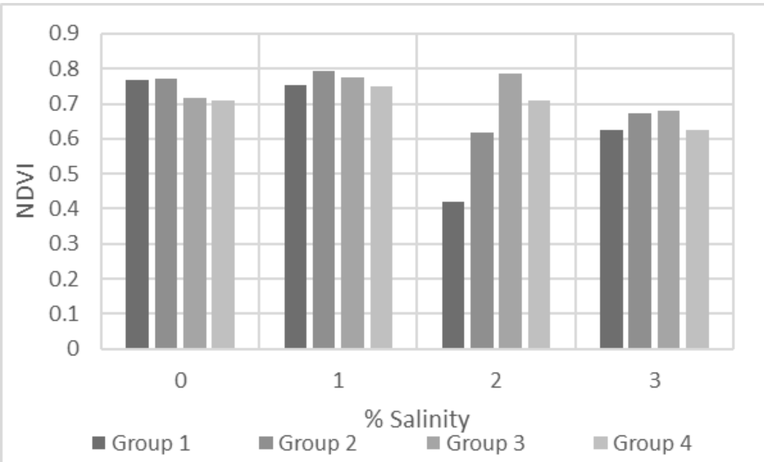

Figure 12. NDVI week 1

Figure (12) shows the NDVI data from week 1 and it includes all 4 groups. Almost all the samples were above 0.6 which indicated a healthy vegetation level, however, the average from group 1 in 2% salinity was less than 0.5 which indicates that the vegetation was sparse. When the NDVI values are low such as the one from group 1 in 2% salinity (0.42) it shows that the plant is stressed.

Table 5. NDVI in week 2 of phaseolus vulgaris in different growth conditions with bacillus subtilis

	Treatment	T1 NDVI	T2 NDVI	Average
Group 1 (Seeds germinated in Bacillus subtilis)	0g/mL	0.70	0.70	0.70
	1g/mL	0.55	0.74	0.65
	2g/mL		0.61	0.61
	3g/mL	0.66	0.37	0.52
Group 2 (Seeds inoculated with Bacillus subtilis)	0g/mL	0.65	0.73	0.69
	1g/mL	0.74	0.71	0.72
	2g/mL	0.53	0.65	0.59
	3g/mL	0.56	0.53	0.55
Group 3 (Bacillus Subtilis Spread on Soil)	0g/mL	0.69	0.64	0.66
	1g/mL	0.70	0.63	0.66
	2g/mL	0.67	0.61	0.64
	3g/mL	0.49	0.55	0.52
Group 4 (No Bacillus Subtilis)	0g/mL	0.69	0.72	0.70
	1g/mL	0.73	0.74	0.73
	2g/mL	0.69	0.74	0.71
	3g/mL	0.70	0.49	0.60

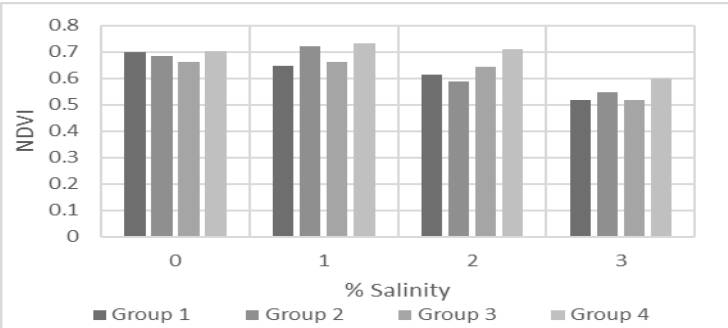

Figure 13. NDVI week 2

Figure (13) shows the NDVI data from week 2 and it includes all 4 groups. Almost all the samples were above 0.6 which indicated a healthy vegetation level; however, the values were lower in all of the four groups at 3% salinity. This shows that the higher the salinity is, and 3% is the highest level in this experiment, the NDVI is lower which indicates that the plant is not as healthy as it should be.

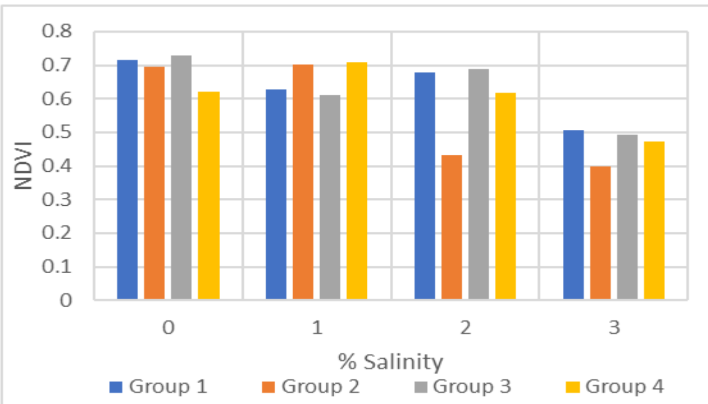

Figure 14. NDVI week 3

Table 6. NDVI in week 3 of phaseolus vulgaris in different growth conditions with bacillus subtilis

	Treatment	T1 NDVI	T2 NDVI	Average
Group 1 (Seeds germinated in Bacillus subtilis)	0g/mL	0.72	0.71	0.71
	1g/mL	0.53	0.73	0.63
	2g/mL		0.68	0.68
	3g/mL	0.50		0.50
Group 2 (Seeds inoculated with Bacillus subtilis)	0g/mL	0.69	0.69	0.69
	1g/mL	0.69	0.71	0.70
	2g/mL	0.49	0.37	0.43
	3g/mL	0.44	0.36	0.40
Group 3 (Bacillus Subtilis Spread on Soil)	0g/mL	0.74	0.71	0.73
	1g/mL	0.71	0.51	0.61
	2g/mL	0.67	0.70	0.69
	3g/mL	0.51	0.48	0.49
Group 4 (No Bacillus Subtilis)	0g/mL	0.69	0.55	0.62
	1g/mL	0.70	0.72	0.71
	2g/mL	0.52	0.72	0.62
	3g/mL	0.50	0.45	0.47

Figure (13) shows the NDVI data from week 2 and it includes all 4 groups. Almost all the samples were above 0.6 which indicated a healthy vegetation level; however, the values were lower in all of the four groups at 3% salinity. This shows that the higher the salinity is, and 3% is the highest level in this experiment, the NDVI is lower which indicates that the plant is not as healthy as it should be.

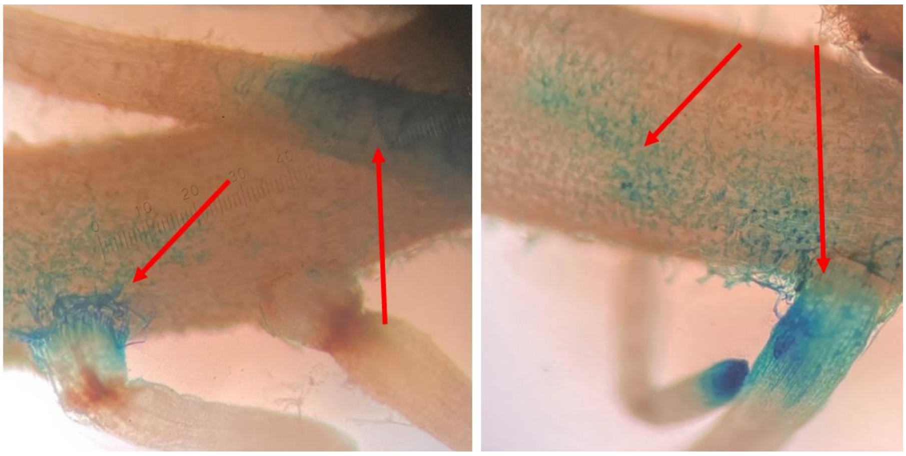

Figure 15. Roots inoculated with b. sub showed signs of arbuscular mycorrhizal fungi

When checking the roots with Bacillus Subtilis under the microscope observations were made and it appeared that some of the roots showed signs of Arbuscular Mycorrhizal (AM) fungi. Based on this observation and some literature review it showed that B. subtilis could lead to AM fungi on plant roots. Literature review stated that B. subtilis had a positive synergism with arbuscular mycorrhizal fungi (Kohler et al., 2007). According to Hashem et al., (2019) the plants which were treated with high salinity levels showed an increase in tannin, ash and lignin content, moreover, the total lipids were reduced in contrast to the control. However, those losses were reduced in the presence of B. subtilis and AM fungi, and they appeared to have improved nutrients absorption in the presence of high salinity levels.

Discussion

The measurements this study focused on were the germination stage, shoot height, and the normalized difference vegetation index (NDVI). Two of those measurements were taken using the phenospex and the germination stages were measured by observation due to the lack of equipment. The overall data for the shoot height showed that Group 1—using the germination in b. sub method—had the best results when it comes to the shoot height. The highest average in week 3 reached 25.3 cm for 0% salinity and the lowest one was in the 3% salinity which was 12.7 cm. In contrast, group 4 in 0% salinity was 17.6 cm and in 3% salinity it showed a sharp reduction in the shoot height and the average reached 5.4 cm. Moreover, group 2 highest height reached 23.67 cm in 0% salinity and showed the same sharp reduction in 3% salinity which reached 6.88 cm. Group 3 had similar results; 0% salinity height average reached 22.7 cm, and 3% salinity reached 4.8 cm. This part of the data analysis indicates that the best method to introduce b. sub to plants would be germination in the bacterial solution. Next, we have the NDVI data; The NDVI index ranges from -1.0 to 1.0. Values below 0.1 indicate bare soil, 0.1 – 0.5 are sparse vegetation, and dense vegetation ranges from 0.6 and above. The data obtained from this measurement indicated that the higher the salinity is, and 3% is the highest level in this experiment, the NDVI is lower which indicates that the plant is not as healthy as it should be. However, when it comes to the plants with bacteria their NDVI levels were slightly more elevated in contrast to the group without bacteria. The literature review stated that B. Subtilis are PGPR's and they have the mechanism to resist abiotic stresses. Therefore, group 1, 2, and 3 had better growth in comparison to group 4 which had no bacteria. According to Shaharoona et al., (2008) Some PGPR include an enzyme called 1-aminocyclopropane-1-carboxylate (ACC)-deaminase, which hydrolyzes ACC (an immediate precursor of ethylene in higher plants) in the roots to ammonia and -ketobutyrate. When these bacteria are present near the root, they significantly reduce ethylene production in roots, promoting root development. Because ethylene production in roots may vary depending on the nutritional condition of the roots and/or rhizosphere, it is extremely likely that the efficacy of PGPR containing ACC-deaminase to boost growth of inoculated plants may vary significantly depending on fertilizer application rates. Moreover, Bacillus Subtilis restricts the uptake of Na ions in the roots with cutting the flow of sodium; this happens on the roots that were covered by PGPR's and in this case, bacillus subtilis. This study showed that when the roots are covered with bacillus subtilis the plant can resist and adapt to high salinity levels and continue growing normally, and in certain cases more than the plants without bacillus subtilis. Moreover, when looking into the last set of results obtained and when we look into Image (19) we can notice a growth of AM fungi on the roots. According to Hashem et al., (2019) plants exposed to high salt levels exhibited an increase in tannin, ash, and lignin content, whereas total lipids were decreased in comparison to the control. However, in the presence of B. subtilis and AM fungi, those losses were decreased, and they appeared to increase nutrient absorption in the presence of high salt levels.

Conclusion

The aim of this study was to assess the effect of the bacterial inoculation method for saline soils and how it improves crop growth in the middle east, specifically, the United Arab Emirates. As UAE has adverse condition for the agriculture, rain doesn't fall often for the agriculture. It has also severe situation of soil salinity. For overcoming these problems, we must take the help of nature. Nature has also provided solution in form of bacillus subtills. Bacillus subtills comes under genus bacillus and in the member of halophytes. Studies show that bacillus subtilis helps plant to maintain its proper metabolic actions properly such as respiration, intake, and exchange of gaseous, chlorophyll percentage etc. Studies also suggest that those plants who were kept with bacterium-bacillus subtills vs. without bacillus subtilis, it was found that bacillus subtilis positive plants were yielded more than those plants without of bacillus subtilis. This can help in solving the severe problem of UAE i.e., is agriculture. They have specific biochemistry, morphology, and physiology that allows them to survive in harsh conditions. Since using of these biotic agents we can also plays a vital role in reducing the human health risk as well as environmental risk, and by promoting the biotic agents for agriculture, we can be the pioneer in new methods of farming. According to the author Grover, M., & Sandhya, V., (2010) A wide range of adaptations and mitigation strategies are required to cope with such impacts. Efficient resource management and crop/livestock development for developing superior breeds can assist to some extent overcome abiotic stressors. The use of these microbes in and of themselves can ease stressors in agricultural plants, bringing up a new and rising use in agriculture. These microbes also provide excellent models for understanding the stress tolerance, adaptation, and response (Grover & Sandhya, 2010). This study confirmed that using Bacillus Subtilis with Phaseolus Vulgaris indeed increases its resistance in saline conditions. the methods which showed the highest yield and had the healthiest set of plants were of group (1); germination of seeds in Bacillus subtilis, and group (2); inoculation of seeds with bacillus subtilis. These bacteria are plant growth promoting rhizobacteria and halophilic bacteria; they help in promoting growth against abiotic stressors and they grow in high salt concentrations. Therefore, using this type of bacteria in saline soils could help improve crop yield and enhance agricultural practices. Moreover, using this bacterium could be the solution to agricultural problems due to high salinity levels if they were engineered and modified to enhance their resistance tolerance. The recommendations to this study would be to implement it in a controlled field and explore the usage of Bacillus Subtilis with different plants and soils as well as to check how other factors, such as temperature, affect the growth of plants in saline conditions while Bacillus Subtilis is present in that environment. Another recommendation would be collecting data with different methods and using DNA sequencing to check how Bacillus Subtilis affect soils and the microbes that exist in those soils.

References

Abdelfattah, M. A., & Pain, C. (2012). Unifying regional soil maps at different scales to generate a national soil map for the United Arab Emirates applying digital soil mapping techniques. *Journal of Maps, 8*(4), 392-405.

Ashrafuzzaman, M., Hossen, F. A., Ismail, M. R., Hoque, A., Islam, M. Z., Shahidullah, S. M., & Meon, S. (2009). Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *African Journal of Biotechnology, 8*(7).

Böer, B. (1999). *Ecosystems, anthropogenic impacts and habitat management techniques in Abu Dhabi.* Fach Geographie, FB 1, Univ. Paderborn.

EAD. (2009). *Soil survey of Abu Dhabi Emirate-extensive survey*.

EAD. (2012). *Soil survey of the Northern Emirates. A set of 3 volumes and maps*.

Ferreira, N. C., Mazzuchelli, R. de, Pacheco, A. C., Araujo, F. F., Antunes, J. E., & Araujo, A. S. (2018). Bacillus subtilis improves maize tolerance to salinity. *Ciência Rural, 48*(8). https://doi.org/10.1590/0103-8478cr20170910

Grover, M., Ali, S. Z., Sandhya, V., Rasul, A., & Venkateswarlu, B. (2011). Role of microorganisms in adaptation of agriculture crops to abiotic stresses. *World Journal of Microbiology and Biotechnology, 27*(5), 1231-1240. http://doi.org /10.1007/s11274-010-0572-7

Harwood, C. R. (1992). Bacillus subtilis and its relatives: Molecular biological and industrial workhorses. *Trends in Biotechnology, 10*, 247–256. https://doi.org/10.1016/0167-7799(92)90233-l

Hashem, A., Tabassum, B., & Abd_Allah, E. F. (2019). Bacillus subtilis: A plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi Journal of Biological Sciences, 26*(6), 1291-1297.

Lastochkina, O., Aliniaeifard, S., Garshina, D., Garipova, S., Pusenkova, L., Allagulova, C., ... & Sobhani, M. (2021). Seed priming with endophytic Bacillus subtilis strain-specifically improves growth of Phaseolus vulgaris plants under normal and salinity conditions and exerts anti-stress effect through induced lignin deposition in roots and decreased oxidative and osmotic damages. *Journal of Plant Physiology, 263*, 153462.

Lopez-Alvarez, B., Ramos-Leal, J. A., Morán-Ramírez, J., & Arango-Galvan, C. (2021). Edaphological and water quality conditions that limit agricultural development in semi-arid zones of Northeastern Mexico. *Environmental Monitoring and Assessment, 193*(1), 1-17.

Kohler et al., J. Kohler, F. Caravaca, L. Carrasco, A. Roldan (2007) Interactions between a plant growth-promoting rhizobacterium, an AM fungus and a phosphate-solubilising fungus in the rhizosphere of Lactuca sativa. *Appl. Soil Ecol., 35* (3) (2007), pp. 480-487

Machado, R., &Serralheiro, R. (2017). Soil salinity: Effect on vegetable crop growth. management practices to prevent and mitigate soil salinization. *Horticulturae, 3*(2), 30. https://doi.org/10.3390/horticulturae3020030

Mahejibin, K., & Patel, C. B. (2007). Plant growth promoting effect of Bacillus firmus strain NARS1 isolated from Central Himalayan region of India on Cicer arientnum at low temperature. In *8th African Crop Science Society Conference, El-Minia, Egypt, 27-31 October 2007* (pp. 1179-1181). African Crop Science Society.

Malik, K. A., Bilal, R., Mehnaz, S., Rasul, G., Mirza, M. S., & Ali, S. (1997). Association of nitrogen-fixing, plant-growth-promoting rhizobacteria (PGPR) with kallar grass and rice. In *Opportunities for Biological Nitrogen Fixation in Rice and Other Non-Legumes* (pp. 37-44). Springer, Dordrecht.

Martinez, R. M. (2013). *Bacillus subtilis. Brenner's encyclopedia of genetics* (Second ed., pp. 246-248). Elsevier Inc. https://doi.org/10.1016/B978-0-12-374984-0.00125-X

Mishra, M., Kumar, U., Mishra, P. K., & Prakash, V. (2010). Efficiency of plant growth promoting rhizobacteria for the enhancement of Cicer arietinum L. growth and germination under salinity. *Adv Biol Res, 4*(2), 92-96.

Municipality, D. (2005). Satellite imagery and thematic mapping project. Executed jointly by Global Scan Technologies (Dubai) and National Remote Sensing Agency, 221.

Naz, I., Bano, A., & Ul-Hassan, T. (2009). Isolation of phytohormones producing plant growth promoting rhizobacteria from weeds growing in Khewra salt range, Pakistan and their implication in providing salt tolerance to Glycine max L. *African Journal of Biotechnology, 8(*21).

Rao, N. K., Shahid, M., & Shahid, S. A. (2009). Alternative crops for diversifying production systems in the Arabian Peninsula. *Arab Gulf Journal of Scientific Research*, *27*(4), 195-203.

Shahid, S. A., & Abdelfattah, M. A. (2008). *Soils of Abu Dhabi Emirate*. Terrestrial environment of Abu Dhabi Emirate. Environment Agency, Abu Dhabi, 71-91.

Shahid, S. A., Zaman, M., & Heng, L. (2018). Introduction to soil salinity, sodicity and diagnostics techniques. In *Guideline for Salinity Assessment, Mitigation and Adaptation Using Nuclear and Related Techniques* (pp. 1-42). Springer, Cham.

Shaharoona, B., Naveed, M., Arshad, M., & Zahir, Z. A. (2008). Fertilizer-dependent efficiency of Pseudomonads for improving growth, yield, and nutrient use efficiency of wheat (Triticum aestivum L.). *Applied Microbiology and Biotechnology, 79*(1), 147-155.

Author Information

Shamma Alghafri
Zayed University
Abu Dhabi, United Arab Emirates
Contact e-mail: *shamma.m.alghafri@gmail.com*

To cite this article:

Alghafri, S. (2022). Exploring the use of bacillus subtilis to improve the growth of phaseolus vulgaris under saline conditions. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 5*, Pages 12-27.