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RESEARCH PAPER

Isolation and identification of paenibacillus sp.from rhizospheric soil and its effect on barley growth

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A B S T R A C T:
Potassium is one of the most important elements in plant growth and development. Most of the potassium reserves on earth are in insoluble mineral form, Potassium solubilizing bacteria (KSB) have been found to dissolve potassium from insoluble K-bearing minerals. In this study, bacterial isolates were obtained from rhizosphere zone, has the same conditions where cultured on Aleksandrov medium containing mica muscovite mineral powder used as k-source to selected KSB, Isolates were purified and identified. Ten isolates of (KSB) diagnosed *Paenibacillus sp*. KSB3 isolate had larger value of SI=7.82 and KSB7 lower value SI=4.00, (61.00, 33.00)mg.ml^{-1} in broth medium for KSB3 and KSB7 respectively, SI gave highly significant positive r= 0.993**with K amount in broth, barley plant was used as indicator to determine the KSB (biofertilizer) efficiency and organic fertilizers effects on plant length, leaf (length, width, area, number), tiller number and stem diameter, leaf (length, width, area) gave significant results with biofertilizer. The values of length plant were highly significant positively correlated with leaf (length, width, area), r= 0. 871**,0.793**, 0.806 ** respectively. Both fertilizers had significant influence on potassium availability in soil.

KEY WORDS: Rhizosphere Bacteria, Potassium Solubilization, Mica Powder, Paenibacillus sp, Sorghum plant.
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1. INTRODUCTION:

As a whole grain, barley (Hordeum vulgare L.), a member of the grass family, contains fiber, vitamins, and minerals. It is a cereal grain that may be used to make bread, beverages, stews, and other foods. According to the 2010 American Dietary Guidelines, they provide a number of health advantages. The soil's quality must be improved, especially with regard to the plant-essential elements potassium, phosphorus, and nitrogen. The third most significant mineral nutrient that limits plant growth is potassium. It is crucial for the metabolic functions of plants, including photosynthesis, respiration, macromolecular biosynthesis, and disease resistance. Bio-fertilizers have been used to grow crops for decades in order to provide them with nutrients, stimulate plant development by producing plant hormones, limit the activity of plant diseases, and improve soil. Building (Abdel-Salam and Shams, 2012). To lessen the need of chemical fertilizers, bio-fertilizers are applied to the soil or plant (Archana et al. 2017). Some varieties of rhizosphere bacteria are able to easily dissolve potassium compounds from the soil, which is a crucial role played by microorganisms in the nature of the potassium cycle. Bacteria that metabolize potassium dissolve potassium. Specifically, aluminum and silicon from potassium-bearing minerals like orthoclases that are insoluble, Mica and illite Paenibacillus polymyxa, formerly known as Bacillus polymyxa, is a Gram-positive, spore-forming, rod-shaped bacterium that has many advantageous properties for agricultural use (Saeed et al., 2021: Zhai et al., 2021).

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This is accomplished by organic acids that dissolve potassium rock or chelated silicon ions to release potassium in solution (Mahendra et al., 2016). By improving the availability of nutrients, such as potassium solubilization, and by creating chemicals that can improve soil structure and fertility, it is a free-living organism that can improve soil health. Additionally, it can encourage plant growth by promoting drought resistance, nitrogen uptake, and root formation. Additionally, it functions as an organ and a soil conditioner fertilizer to digest contaminants in growing media, such as heavy metals and polycyclic aromatic hydrocarbons. (Sheng and He 2006). The formation of organic acids such oxalic acid and tartaric acid, as well as the production of capsular polysaccharides that aid in the dissolution of minerals by the solubilizing illite and feldspar mediated microorganisms (Ahmad et al., 2016; Ali et al., 2020), The flow of potassium in the soil is influenced by potassium-solubilizing bacteria, which also improve soil preparation for plants. That demonstrates that the breakdown of insoluble potash compounds occurs either by increasing the acidity of the medium or soil due to organic acids produced, which is known as acidolysis, or by the formation of complex compounds in the medium, which is known as complex lysis. The third process, known as the exchange reaction, is responsible for the breakdown of insoluble potash compounds the third mechanism, called the exchange reaction, converts insoluble potassium compounds present in minerals into the soluble form of potassium. It is responsible for breaking down insoluble potash compounds. This causes a rise in the plant's nutritional readiness and promotes cation chelation (Das, 2016), However, frequent and excessive use of chemical fertilizers results in risks to human health and the environment, deterioration of soil qualities, and ultimately a lack of crops (Etesami et al., 2017). Therefore, the current study's objectives were to identify and identify Paenibacillus sp. from the native soil of Halabja Governorate's rhizosphere for various plants (crops and fruits), as well as assess its effectiveness in dissolving potassium compounds in the soil. To explain their impact, peanibacillus sp. isolates were used as bio-fertilizer with barely seeds.

2. MATERIAL AND METHODS

The examined location was Tabaqora village, Sharazoor, and is located in the southwestern extension of the Halabja, Kurdistan region of Iraq. Rhizosphere soil samples were obtained from 15 different plants (fruits, crops), and the area was observed. For the purpose of isolating the bacterium Paenibacillus polymyxa, samples were also taken from 30 and 50 horizontal distances and 30 and 50 vertical distances, as shown in diagram (1). The area in question is located at 35.170581 and 45.974956 in longitude.

R(0)cm → H(30) → cm H(50)cm

↓

V(30)cm

↓

V(50)cm

Diagram (1). Sampling scheme: R: Rhizosphere; V: vertical; H: horizontal

The samples were transferred to the laboratory in sterile plastic bags and kept there until the isolation and diagnosis procedures were completed. The Alexandrov medium, which contains 5.0% glucose, 0.05% MgSO4.7H2O, 0.0006% FeCl3, 0.01% CaCO3, 0.2% Ca3PO4, and 0.3% mica (a source of potassium), was developed to isolate bacteria that dissolve potassium compounds (Parmar and Sindhu, 2013). A series of soil dilutions from 10^{-1} to 10^{-6} have been prepared, and the media have been sterilized using an autoclave device at 121 C^0 temperature and 1.5 pounds inches of pressure for 20 minutes. After that, 1 ml of the dilution (10^{-6}) was transferred and spread using a sterilizer on the moldable Alexandrov medium in the plates before being incubated at (30±1°C) temperature for 7 days, potassium solubilizing bacteria was observed by the formation of a transparent halo around developing colonies on the plates, which indicates the dissolution of the source of insoluble potassium in the medium. The colonies were then selected, purified several times on the plates, and stored in the refrigerator at a temperature of 4 C until use. The culture properties of bacteria and their response to the color of the gram alongside the bacteria movement were examined (Atlas et al., 1995).

2.1 Determination of Solubilization Index (SI) and Potassium Solubilization Efficiency (SE)

For this purpose, Alexandrov solid medium was prepared in Petri dishes and allowed to harden. The medium was then transferred 0.1 ml by means of a sterile pipette of dilution 10-6 of each isolate and spread on the surface of the medium using L-shape diffuser for three repeaters. Dishes were then incubated at a temperature of (30±1°C). After seven days of incubation, using a measurement ruler and the following calculation to estimate the coefficient of dissolution, measure the diameter of the colonies and the translucent halo. (2): Potassium Solubilization Efficiency (SE) = (Solubilization ring diameter, mm) / (colony diameter, mm). (1): SI= (Colony diameter + potassium solubilizing ring diameter, mm)/ (colony diameter, mm) × 100. (Mahendra et al., 2016).

2.2 Identification of Bacteria

2.2.1 Culture Tests:

The phenotypic characteristics of the developing colonies were observed in terms of their shapes, colors, surfaces, and edges, the presence of distinctive odors, their transparency, and consistency on the nutrient agar, in order to diagnose Paenibacillus sp. All the selected isolates were examined for the colony phenotypic characteristics of the developing colonies**.** (Collee et al., 1996).

A- Motility Test: After being incubated at a temperature of 301°C for 24 hours, test tubes containing semi-solid nutritional agar medium were infected with bacteria using the stabbing method. The spread of growth outside the stabbing region is an indication of the isolate's ability to migrate. (Collee et al., 1996).

B-Starch Hydrolysis: As described in (Atlas et al., 1995).

2.2.2 Microscopic Tests:

A- Gram stain: Under the oil microscope's 100 x zoom lens, the forms, arrangements, and interactions of the cells with the dye were observed**.** (Atlas et al., 1995).

B- Capsule staining: This test was done according to (Collee et al., 1996).

2.2.3 Biochemical Tests: Included the following tests: the methyl red test, the oxidase test, the Voges-Proskauer test, the use of citrate as a carbon source, the urease test, the catalase test, the hydrolysis of gelatin, the synthesis of indole, the hydrolysis of starch, the hydrolysis of casein, and the H2S production test, according to the findings in. (Atlas et al., 1995).

Quantitative estimation of K released from insoluble K bearing minerals

Bacterial isolates' capacity to release K from broth media that has been treated with 1% muscovite mica. One milliliter of the overnight cultures of each isolate were transferred to 25 ml of Aleksandrov broth in three replicates to dissolve potassium in the liquid culture medium to which 1% of mica mineral was added. Three weeks were spent incubating each of the inoculation flasks at (30±1°C). In comparison to a group of uninoculated controls, the amount of K released in the broth was calculated after 20 days of incubation from triplicate flasks at each stage. To separate the supernatant from the cell growth and insoluble potassium, the broth cultures were centrifuged at 6000 rpm for 20 minutes. Flame photometry was used to determine the supernatant's available K content. (Sugumaran and Janarthanam, 2007) a standard curve was prepared using various concentrations of: 0, 10,20, 30, 40 to 100 ppm ($mg.L^{-1}$) solution. The amount of potassium solubilized by the isolates was calculated from the standard curve.

3. POT EXPERIMENT

Pot experiment was performed in a Factorial Completely Randomized Design (FCRD), in pots filled with 5 kg of Silty clay loam soil. Barley seeds were used in the pot experiment (2 × 2 × 3), and the total duration of the experiment was 30 days. A pot experiment was conducted in the greenhouse of Salahaddin University, Agriculture Engineering Sciences in order to check the effect of KSB on barley crop growth. Barley seeds were surface disinfected by immersing in 3% (v/v) sodium hypochlorite for 5 min. After that, Barley seeds were divided in to two parts, first part were extensively washed with sterilized water 3 times for 5 min. Following treated with sugar solution for few minutes, next, seeds were inoculated by immersion in a culture containing 4.6×109 CFU /ml^{-1}. Peanibacillus polymyxa (predominant bacteria isolate as represented by higher K solubilizing efficiency on the isolation plates were selected) for 30 min. (Pure isolates of *Paenibacillus polymyxa* inoculated in broth media and incubated at (30±1°C) for 18 hours ,15 seeds were sown per pot, then thinned into 10 plants/pot. Negative controls seeds were not inoculated with bacteria and didn't add bitmoss to pot soil. In this experiment, bitmoss was used for the purpose of carry bacterial Inoculation (was sterilized in121 C^{0}60 min.) to get rid of microorganisms (Elkoca et al., 2013). Mineral fertilizer was added as fertilizer recommended full recommendation to each of nitrogen and phosphorous while ½ for potassium), Experiment factors included four treatments: biofertilizer (B0, B1), organic fertilizer (O0, O1), Bitmoss 12.5gm for 5kg of soil as shown in table 1.

Table (1): Explains different treatments of pot experiment

No.	Treatment	Symbol
T1	B_0O_0	Control
T2	B0O1	Without bacteria + with bitmoss
T3	B_1O_0	With (bacteria) Pp+ without bitmoss(Pp:Paenibacillus polymyxa)
T4	B_1O_1	With (bacteria) Pp + with bitmoss

4. SOIL PHYSICOCHEMICAL PARAMETERS

The soil samples used in the pot experiment were air dried, pulverized, and sieved using a 2 mm sieve. Physiochemical properties were examined using the established techniques Burt outlined (Burt, 2004). Using a pH meter, the soil pH in the soil suspension was obtained, and the ECe was calculated using (Hesse, 1972) method, Total calcium carbonate Soil (Hesse, 1972) method, organic matter determined by Walkely and black method (Jackson, 1973). Total nitrogen was measured by Kjeldahl’s method (Subbaiah and Asija, 1966). Available phosphorus was determined by Olsen’s method (Muhr et al., 1965). Potassium from the soil that is both available and soluble was extracted using ammonium acetate and quantified using a flame photometer (Stanford and English, 1949). Titrimetry was utilized to measure carbonate and bicarbonate, and a hydrometer was used to assess the soil's texture. (Burt, 2004), tab. 2 displayed some soil characteristic:

Table (2): Some physical and chemical properties of the studied soil before planting*.

Properties	Value		Properties	Value
Bulk density gm.cm^{-3}	1.70	**Soluble ions Cmol.Kg^{-1}**	**Cations**	
ECe ds.m^{-1}	0.98		Ca^{+2}	0.318
Soil pH	7.85		Mg^{+2}	0.122
SOM gm.kg^{-1}soil	12.50		Na^{+1}	0.095
$CaCO_3$ gm.kg^{-1} soil	265.50		K+1	0.066
Total N. mg. g^{-1}soil	1.85		Anions	
Available P. µg.gm-1soil	2.62		HCO3-1	0.5865
Total K. gm.kg^{-1} soil	7.8		CO3^{-2}	0
Soil texture (Silty clay loam)			Cl-1	0.242
Sand gm.kg-1	13		SO_4-2	0.580
Silt gm.kg-1	51			
Clay gm.kg-1	36			

*ECe: electrical conductivity, P: phosphorous, K: potassium, N: nitrogen, SOM: soil organic matter.

5. EXPLANATIONS: Explanations on plant growth parameters were recorded at 30 days after sowing.

6. PLANT GROWTH PARAMETERS

Plant length, Stem diameter, Tiller number, leaf number, leaf length, leaf width, leaf area.

7. STATISTICAL ANALYSIS

The data were analyzed statistically according to the methods variance analysis (ANOVA), Duncan's and simple correlation coefficient. (Steel and Torrie, 1980).

8. RESULT AND DISCUSSION

8.1 Isolation and Diagnosis of KSB

Potassium-soluble bacterial isolates were obtained by producing clear zone around developed colony on Aleksandrov agar medium, the diagnostic results were shown among 37 bacterial isolates, 10 bacterial isolates were found belonging to the genus Paenibacillus polymyxa, 9 isolates were found in the Rhizosphere area, except for one were found in V(50)cm region, Paenibacillus polymyxa isolates coded KSB-1 to KSB-10. The morphological characterization, microscopic and biochemical tests displayed in tab. (3,4).

Table (3): Colony morphological and microscopic tests of the KSB isolates

N.	KSB Isolates	Morphological characters and microscopic tests		
		Colony characters	Gram test & cell shape	Capsule test
1-	KSB1	Transparent, circular white, small	G+ve , Rod	+
2-	KSB2	Transparent, white, convex	G+ve , Rod	+
3-	KSB3	Transparent, circular white, small	G+ve , Rod	+
4-	KSB4	Transparent, circular white, small	G+ve , Rod	+
5-	KSB5	Transparent, white, small	G+ve , Rod	+
6-	KSB6	Transparent, white, small	G+ve , Rod	+
7-	KSB7	white, small, round	G+ve , Rod	+
8-	KSB8	Transparent, circular white, small	G+ve , Rod	+
9-	KS9	Transparent, circular white, small	G+ve , Rod	+
10-	KSB10	Transparent, white, small	G+ve , Rod	+

Table (4): Biochemical characterization* and identification of the KSB

Biochemical Tests														Genus obtained
N.	KSB	OX	VP	CA	ST	In	U	CI	M.R	C	M	g	G	TSI	
1-	KSB1	-	-	-	+	-	-	+	-	+	+	-	-	K/N	Paenibacillus
2-	KSB2	-	-	-	+	-	-	+	-	+	+	-	-	A/A	Paenibacillus
3-	KSB3	-	-	-	+	-	-	+	-	+	+	-	-	K/N	Paenibacillus
4-	KSB4	-	-	-	+	-	-	+	-	+	+	-	-	K/N	Paenibacillus
5-	KSB5	-	-	-	+	-	-	+	-	+	+	-	-	A/A	Paenibacillus
6-	KSB6	-	-	-	+	-	-	+	-	+	+	-	-	A/A	Paenibacillus
7-	KSB7	-	-	-	+	-	-	+	-	+	+	-	-	A/A	Paenibacillus
8-	KSB8	-	-	-	+	-	-	+	-	+	+	-	-	K/N	Paenibacillus
9-	KS9	-	-	-	+	-	-	+	-	+	+	-	-	K/N	Paenibacillus
10-	KSB10	-	-	-	+	-	-	+	-	+	+	-	-	A/A	Paenibacillus

*OX – oxidase, V.P. –Voges proskauer, CA– Casein hydrolysis, ST– Starch hydrolysis, In – indol test, U– Urea hydrolysis, CI– Citrate utilization, MR– Methyle red test, M–motility, H2S– H2S production, g– Gas production, G– Gelatin liquefaction, TSI– carbohydrate fermentation, C: Catalase, + : Positive, - : Negative.

8.2 Screening the Ability of Bacterial Isolates to Solubilize Potassium

As a result of variations in the diameters of the transparent zones around bacterial colonies on the surface of solid culture of Alexandrov media, which range in size from 4.00 to 7.82 mm, the results revealed variance in isolates' capacities to dissolve potassium mineral (mica) during the incubation period's. The findings revealed that isolate KSB-3 had the highest solubilization index (7.82mm), while isolate KSB-7 had the lowest (4.00mm), as shown in tab. 5.

Table (5): Details of solubilization index of KSB isolates

N.	KSB Isolates	Colony diameter(d)mm	Zone diameter(D)mm	Solubilization Index(SI)	K-concentration 20day(µg/ml)	SE (SF, %)
1-	KSB$_1$	2.10	9.90	5.71	44.07	471.43
2-	KSB$_2$	2.10	11.90	6.67	53.50	566.67
3-	KSB$_3$	2.30	15.70	7.82	61.00	682.61
4-	KSB$_4$	1.80	8.80	5.88	44.84	488.43
5-	KSB$_5$	2.00	8.30	5.15	41.88	415.00
6-	KSB$_6$	1.70	6.30	4.71	38.22	371.13
7-	KSB$_7$	1.50	4.50	4.00	33.00	300.00
8-	KSB$_8$	2.20	12.10	6.50	52.80	550.82
9-	KSB$_9$	2.00	10.50	6.25	49.50	525.00
10-	KSB$_{10}$	2.00	9.00	5.50	43.77	450.00

8.3 Determination of the Amount of Dissolved Potassium in Broth Media For the isolates that had a zone of solubilization on Aleksandrov agar medium, additional tests were carried out to determine how well they could release K from broth media. The findings indicated that the isolates released between (33.00 to 61.00) g.ml-1 of soluble potassium from potassium mineral (mica) in the broth at 20 days after incubation under laboratory conditions. The solubilization was highest in KSB3, with KSB2 coming in second with 53.50 g/ml, KSB8 coming in second with 52.80 g/ml, and KSB9 coming in at 49.50 g/ml. Two isolates, KSB6, KSB7, gave (38.22, 33.00) g.ml-1 of potassium solubilization from muscovite mica, whereas four isolates demonstrated (44.84, 44.07 43.77, 41.88) g.ml-1 KSB4, KSB1, KSB10, and KSB5 correspondingly. According to statistical analysis, there is a highly significant positive correlation between potassium concentration in broth and each of SI, SE, D, and d. The correlation coefficients (r) between potassium concentration in broth and SI and SE were 0.993 and 0.992, respectively, and between potassium concentration in broth and D and d were 0.985 and 0.883, respectively. The four parameters (SI, SE, D, and d) provided a highly accurate representation of the potassium content in broth; hence, our analysis revealed a favorable association between SI and SE, D, and d, as shown in figure 1(A, B, C, and D), which was followed by d and D. Our result was consistent with the speed of microbial growth and the capacity of microbial metabolism, both of which While disagreeing with (Chiang et al., 2013), who found no correlation between SI value and concentration of potassium solubilizing of broth on all K sources (trachyte, feldspar, leucite), (Chishi, 2010) studied the amount of potassium released from muscovite mica in a broth by the ranged were from 2.41 g/ml to 44.64 g/ml at 20 days and SI value for isolates (SI> 4) was very high. Even while several of their isolates' strains produced clear zones (SI values) when screened on media smaller than 4 mm, they could best solubilize insoluble potassium on those media. Instead, they came to the conclusion that high SI values did not always translate into high K solubilizing, and that potassium-solubilizing indexes do not always correlate with the capacity of microbes to dissolve poorly soluble potassium sources in liquid media. They attributed this finding to the amount of organic acids that isolates produce, which they said is the cause of the discrepancy.

The isolates used in this study are all from the same species and were found in the same soil and environment in a single field. The difference between the isolates is the type of vegetation present, and some isolates may be able to dissolve potassium more effectively than others due to plant organic residues or the decomposition of organic matter, both of which play a significant role in the microbial diversification of one species' strain. (Allison et al., 2007)

The main cause of K's solubilization is the activity of organic acids, both microbial and plant-derived. However, just like all physiological processes, the efficiency of K solubilization is strongly regulated by external variables like temperature or pH and nutrients like sugar type. (Verma et al., 2020). When glucose was substituted with other sugars like galactose, xylose, or arabinose, K solubilization was found to be relatively lower among the isolates in

(Kaur and Kumar's 2018)'s study they explained that different plants produce different sugars, which in turn affects how easily potassium can be dissolved by bacteria isolates that were isolated from various plants. In their study, (Brockett and colleagues 2012) found that the total microbial community and specific microbial groups were significantly higher in soil under trees than in shrubs or herb-vines. They hypothesized that this was because, from herb-vine to shrub to tree, the soil conditions improved and became more conducive to microorganisms because more nutrient resources were provided in the latter. In fact, the soils under trees had higher water content and microbial diversity. While certain isolates can assemble all the mechanisms for solubilizing K, some KSB contribute to the release of K+ from K-bearing minerals through just one or two mechanisms. As a result of slow releases of exchangeable K, released H+ can directly dissolve the mineral K. Other mechanisms include the decomposition of organic materials, which generates ammonia and acids like nitric acid (HNO3) and sulfuric acid (H2SO4), as well as the production of organic and inorganic acids and protons. This causes the pH of the soil to drop.

(Meena et al., 2016). (Ahmad et al., 2016). (They noted that KSB are typically present in all soils, though their number, diversity, and capacity to dissolve potassium vary depending on the soil, climatic conditions, and their capacity to do so. It has also been known that the type and concentration of the organic acid produced by KSB may differ.

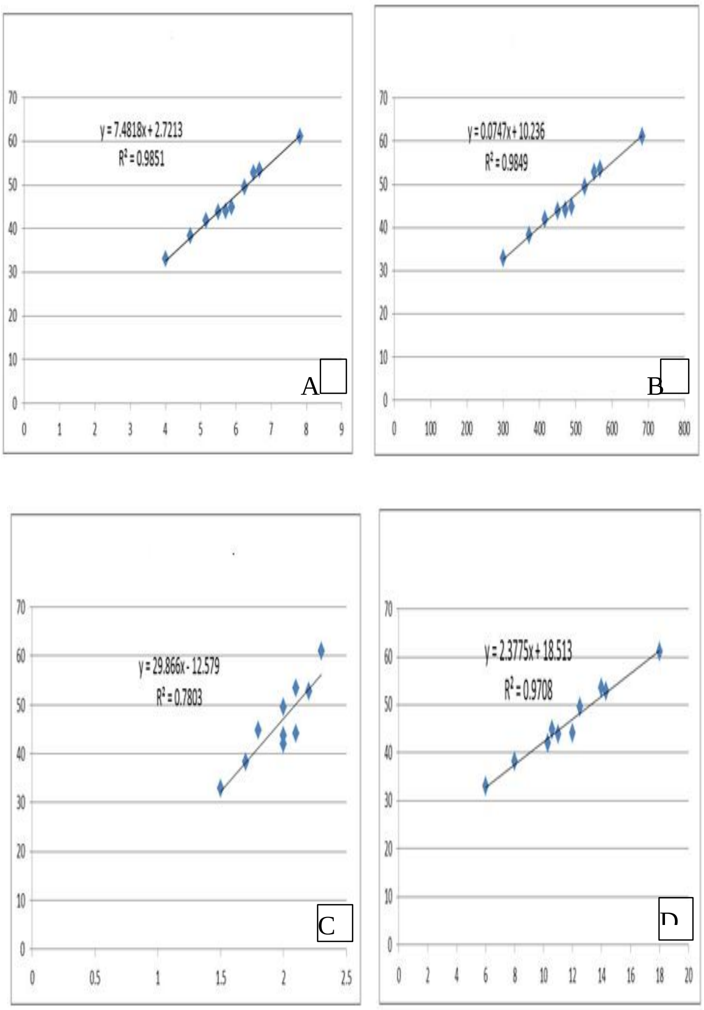

Figure 1: Displays correlation values and simple linear regression equation between K. concentration in broth and SI (A);SE(B);d(C);D(D).

8.4 Effect of Paenibacillus Polymyxa Inoculation and Organic Fertilizer on the Growth of Barley Plant

Paenibacillus polymyxa was chosen for our KSB3 isolate because it had the highest solubilizing zone and K content in both solid and liquid. Alexsandrov conducted a pot experiment in agriculture engineering sciences using a factorial completely randomized design (FCRD) with three replications in order to study the impact of bio fertilizer on the growth of barley plants while using bitmoss as an organic fertilizer. The results of the pot culture experiment were recorded after 30 days in Table 1 of the study.

8.5 Shoot Length

Data regarding plant length (P.L), number of leaves (L.n) per plant and number of tillers (Tiller.n) per plant, tillers diameters (s.d) per plants, leaves length (L.L), width (L.W) and area (L.A) at crop growth stage of 30 days are reported in fig 2 (A,B).

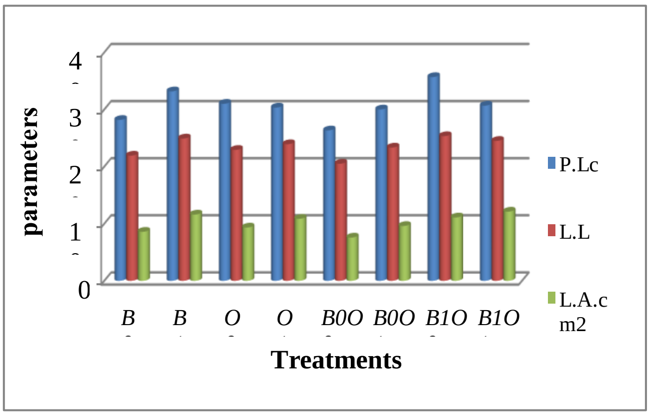

Figure2- A: Effect of (organic and bio) fertilizers on (P.L.,L.L,L.A) growth of barley plant

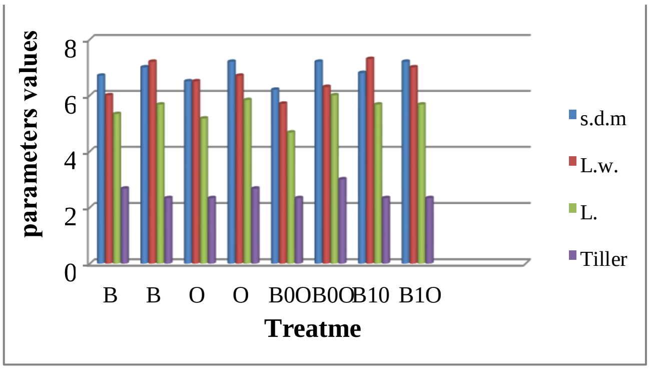

Figure2- B: Effect of (organic and bio) fertilizers on (s.d,L.W,L,n,Tiller.n) growth of barley plant

Application of KSB and organic fertilizers gave maximum P.L (35.67 cm), L.L (25.33)cm, L.W. (0.73)cm in B1O0, L.A.(12.11)cm^2 in B1O1,whereas B0O1 recorded maximum L.n. per plant (6) and tillers n. per plant (3), maximum s.d. (0.72)cm was noted in O1,B0O1 and B1O1, whereas, lowest values were observed in B0O0 P.L.(26.33)cm, (20.50)cm L.L., L.W.(0.57)cm, L.A.(7.56)cm^2, L.n. (4.67) cm, tiller n (2.33)cm. and steam diameter (0.62)cm .

Statistical analysis of the data revealed that there was a highly significant positive correlation coefficient between plant height with leaf length, leaf width and leaf area, the (r) were 0.871**, 0.793** and 0.806 ** respectively. As well as there was a highly significant positive correlation coefficient between leaf area with leaf length and leaf width(r) given 0.908**and 0.706**, while (r) was positive significant correlation coefficient between Ln. with Tiller and s.d,0.577* and 0.566*,L.L and L.W recorded (r)0.644*

The results analysis of variance (ANOVA) test shown the F values of the bacterial inoculation where F= (5.17*, 5.36*, 5.40* and 6.55*) for the P.L., L.L., L.W. and L.A. respectively tab. (6)

The growth parameters have exhibited interesting variation due to two different fertilizers, similar findings have been reported by several research workers (Keerthi et al., 2018), (Singh et al. 2019) and (Kaur and Kumar, 2018). They attributed the cause to the fact that both types of study fertilizers—organic and biological—supplement the plant with potassium in addition to other fundamental components (P. polymyxa may dissolve phosphorus and fix nitrogen), which promotes the movement of water, nutrients, and carbohydrates in the plant tissue. It has a role in the plant's enzyme activation, which has an impact on the synthesis of protein, starch, and adenosine triphosphate (ATP). Better vegetative development is achieved when the rate of photosynthesis is controlled, which produces ATP.

8.5 Effect of Inoculation of Paenibacillus Polymyxa and Organic Fertilizer on Potassium Forms

Potassium forms in the soil of pot experiments shown in Fig 3, table (7) variance analysis ANOVA shown there were a large differences between the treatments compared to the control the ranged of potassium forms for Av. K were between 450.10mg.kg^{-1} in B0O0 minimum-670.20mg.kg^{-1} maximum, Sol. K(36.55-87.90)mg.kg^{-1} for same treatments and Ex. K gave(413.55-582.28)mg.kg^{-1} in B0O0-B1O1, overall there was a significant relation in treatment bacterial inoculation F=(7.00*) for Av K. soluble k gave F=(8.04*,12.92**) in treatments bitmoss and bacterial inoculation respectively , that F values were significant for B1,O1,the result conclude the existence of the efficiency of isolates under laboratory condition and in practical applications, in addition to the practicality of organic fertilizer, the statistical analysis results a highly significant positive correlation between Av.K and Ex K (r=0.982**) and significant positive correlation between Av.K with Sol.K (r=0.580*) beside Av.K with s. d (r=0.609*), then the positive significant correlation coefficient (r=0.602*) between s.d. and Ex.K.

Paenibacillus spp. have been used and studied as plant growth-promoting agents in agriculture, Paenibacillus polymyxa contains a variety of plant growth hormones, including zeatin , gibberellin, kinetin and auxin , as well as a variety of organic acids, including oxalic acid, tartaric acid, malic acid, lactic acid, acetic acid, citric acid, and succinic acid(Meena et al., 2014). . Qian et al. 2015 study they found the apple seedlings in the paenibacillus polymyxa inoculated group The most effective isolates in the current study were isolated from Fig plants, which may be due to different plant residues and organic residues that can cause changes in the structure and function of microbial communities in the soil. The control group showed more leaves, longer primary roots, higher plant height, and higher dry weight.. (Qian et al. 2015), Microorganisms in the soil are directly involved in the conversion of matter, the release of nutrients and the fixation process are closely related to the quality of the soil environment (Geisseler and Scow 2014), Kong inWhen tobacco plants were watered with isolated K-releasing bacteria in 2014, researchers observed that when compared to the control, plant height, leaf length, and total K intake all increased. The influence and capacity of KSB to manufacture various forms of organic acids, such as (citric, malic, tartaric, and formic), results in diverse KSB methods to K resolving. In general, reducing pH, boosting chelation of the cations attached to K, and acidolysis of the surrounding microorganisms are the three most significant mechanisms recognized in KSB's solubilization of K minerals. (Meena et al., 2014). (Subhashini and Kumar, 2014) Therefore, concerted efforts are made to understand the combined effects of rock material addition and KSB inoculation on nutrient availability in soils and growth of various crops. It has been discovered that potassium nutrients are released slowly from the rock materials, and their use as fertilizer frequently causes insignificant increases in crop yield. Additionally, numerous studies have demonstrated that PGPMs raise growth indicators. (Heredia et al., 2019) the study discovered that barley infected with fluorescent rod-shaped bacteria had increased shoot length, root length, fresh weight biomass, and potassium content in the soil and plant. (Saleem et al., 2019). Additionally, a rod-shaped, fluorescent bacteria that has been strongly demonstrated to be an effective enhancer of the plant barley is also effective in solubilizing potassium under heavy metal stress conditions. This conclusion is in line with our findings about the soluble content.

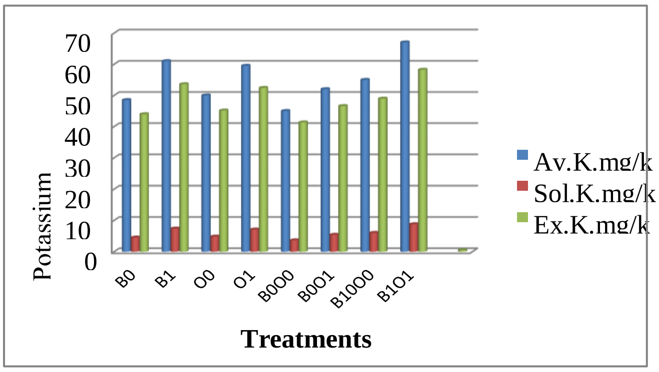

Figure3: Effect of (organic and bio) fertilizers on K (Av,Sol,Ex) forms

Table (6): effect of paenibacillus polymyxa inoculation and organic fertilizers on the growth of Barley plants

Treat.		P.L.	L.L.	L.W.	L.A.	S.D.	L.N	TILLER N.
B	B0	28.17[a]	21.92[a]	0.60[a]	8.58[a]	0.67[a]	5.33[a]	2.67[a]
	B1	33.17[a]	24.92[b]	0.72[b]	11.60[b]	0.70[a]	5.67[a]	2.33[a]
O	O0	31.00[a]	22.92[a]	0.65[a]	9.33[a]	0.65[a]	5.17[a]	2.33[a]
	O1	30.33[a]	23.92[a]	0.67[a]	10.86[a]	0.72[a]	5.83[a]	2.67[a]
B0O0		26.33	20.50	0.57	7.56	0.62	4.67	2.33
B0O1		30.00	23.33	0.63	9.60	0.72	6.00	3.00
B1O0		35.67	25.33	0.73	11.09	0.68	5.67	2.33
B1O1		30.67	24.50	0.70	12.11	0.72	5.67	2.33

- similar letter means non-significant difference

Table (7): effect of paenibacillus polymyxa inoculation and organic fertilizers on concentration of potassium forms

Treat.		Av.K mg.kg^{-1}soil	Sol.K mg.kg^{-1}soil	Ex.K mg.kg^{-1}soil
B	B0	485.15[a]	45.37[a]	439.78[a]
	B1	610.10[b]	74.08[b]	536.03[a]
O	O0	500.05[a]	48.41[a]	451.65[a]
	O1	595.20[a]	71.05[b]	524.15[a]
B0O0		450.10	36.55	413.55
B0O1		520.20	54.19	466.01
B1O0		550.00	60.26	489.74
B1O1		670.20	87.90	582.28

*similar letter means non-significant difference

9. CONCLUSION

The samples were taken from a single location with varying plant coverage; as a result, the bacteria's ability to solubilize potassium varied. The majority of KSB were found in the rhizosphere zone rather than at vertical or horizontal distances from the rhizosphere. The solubilizing index is the most crucial parameter to determine potassium solubilizing, and biofertilizers are more effective than organic fertilizers at increasing soluble and available potassium content in soils.

References

ABDEL-SALAM, M.A. AND SHAMS, A.S., 2012. Feldspar-K fertilization of potato (Solanum tuberosum L.*) augmented by biofertilizer. J Agric Environ Sci, 12(6), pp.694-699.*

AHMAD, M., NADEEM, S.M., NAVEED, M. AND ZAHIR, Z.A., 2016. Potassium-solubilizing bacteria and their application in agriculture. *Potassium solubilizing microorganisms for sustainable agriculture, pp.293-313*.

ALLSION, V.J., CONDRON, L.M., PELTZER, D.A., RICHARDSON, S.J. AND TURNER, B.L., 2007. Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Joset chronosequence New Zealand. *Soil Biol. Biochem, 39, pp.1770-1781.*

ALI, A., ADNAN, M., SAFDAR, M.E, .ASIF, M., MOHAMAD, A., NADEEM, M., JAVID,. M.A, AHMAD, S., QAMAR, R., BILAL, H.M. AND KHAN, B.A, 2020 Roli potassium in enhance growth, yield and quality of maize (zia mays L.).*Int J Biosci, 16(6), pp. 210-19.*

ALKHAFAGI, H.H.A. AND ALNUAIMI, J.J.J., 2023. Effect of Spraying Concentrations of on some Silicon Physiological Characteristics of varieties of Maize by the Effect of Water stress. *Euphrates Journal of Agricultural Science, Volume 1, Issue 15, Pages 20-25.*

ARCHANA, D.S., NANDISH, M.S., SAVALAGI, V.P. AND ALAGAWADI, A.R., 2012. Screening of potassium solubilizing bacteria (KSB) for plant growth promotionalactivity. *Bioinfolet-A Quarterly Journal of Life Sciences, 9(4), pp.627-630.*

BURT, R., 2004. Soil survey laboratory methods manual. Soil survey investigations report 42, Version 4.0. United States Department of Agriculture. *Natural Resources Conservation Service, National Soil Survey*.

CHISHI, K.Y., 2010. Studies on dual inoculation of potassium solubilizing bacteria and phosphorus solubilizing bacteria on growth and yield of maize (Zea mays L.). *Agricultural Microbiology UAS, Dharwad-580005, Karnataka State, India.*

DAS, I. AND PRADHAN, M., 2016. Potassium-solubilizing microorganisms and their role in enhancing soil fertility and health. Potassium solubilizing microorganisms for sustainable agriculture, *pp.281-291.*

DIETARY GUIDELINES ADVISORY COMMITTEE, 2010. Report of the dietary guidelines advisory committee on the dietary guidelines for Americans, 2010. US Department of Agriculture and US Department of Health and Human Services. *Washington, DC: US Government Printing Office, pp.2020-01*.

ETESAMI, H., EMAMI, S. AND ALIKHANI, H.A., 2017. Potassium solubilizing bacteria (KSB):: Mechanisms, promotion of plant growth, and future prospects A review. *Journal of soil science and plant nutrition, 17(4), pp.897-911*.

GEISSELER, D. AND SCOW, K.M., 2014. Long-term effects of mineral fertilizers on soil microorganisms–A review. *Soil Biology and Biochemistry, 75, pp.54-63.*

HEREDIA-ACUÑA, C., ALMARAZ-SUAREZ, J.J., ARTEAGA-GARIBAY, R., FERRERA-CERRATO, R. AND PINEDA-MENDOZA, D.Y., 2019. Isolation, characterization and effect of plant-growth-promoting rhizobacteria on pine seedlings (Pinus pseudostrobus Lindl.*). Journal of Forestry Research, 30(5), pp.1727-1734.*

HESSE, R.O.L.F. AND PER, J., 1972. The Crystal and Molecular Structure of the Gold (I) Dipropyldithiocarbamate Dimer. *Acta Chem. Scand, 26(10).*

HU, X., CHEN, J. AND GUO, J., 2006. Two phosphate-and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. *World journal of Microbiology and Biotechnology, 22(9), pp.983-990.*

JACKSON, M.L., 1958. Soil chemical analysis prentice Hall. Inc., Englewood Cliffs, NJ, 498, pp.183-204.

KAUR, N. AND KUMAR, S., 2018. Effect of tillage and nitrogen scheduling on growth and yield attributes of wheat (Triticum aestivum L.*) in central Punjab. IJCS, 6(1), pp.1666-1669.*

KEERTHI, M.M., BABU, R., VENKATARAMAN, N.S. AND MAHENDRAN, P.P., 2018. Influence of Irrigation Scheduling with Levels and Times of Nitrogen Application on Root Growth of Aerobic Rice. *American Journal of Plant Sciences, 9(11), pp.2297-2305*.

MAZHAR, K.A.R.A., SAYINCI, B., ELKOCA, E., ÖZTÜRK, İ. AND ÖZMEN, T., 2013. Seed size and shape analysis of registered common bean (Phaseolus vulgaris L.*) cultivars in Turkey using digital photography. Journal of Agricultural Sciences, 19(3), pp.219-234.*

MEENA, V.S., MAURYA, B.R., VERMA, J.P. AND MEENA, R.S. EDS., 2016. Potassium solubilizing microorganisms for sustainable agriculture (Vol. 331). *New Delhi: Springer*.

MUHR, G.R., 1965. Soil testing in India.

QIAN, X., GU, J., PAN, H.J., ZHANG, K.Y., SUN, W., WANG, X.J. AND GAO, H., 2015. Effects of living mulches on the soil nutrient contents, enzyme activities, and bacterial community diversities of apple orchard soils. *European Journal of Soil Biology, 70, pp.23-30.*

RAJAWAT, M.V.S., SINGH, S., TYAGI, S.P. AND SAXENA, A.K., 2016. A modified plate assay for rapid screening of potassium-solubilizing bacteria. Pedosphere, 26(5), pp.768-773.Saleem, M., Asghar, H.N., Zahir, Z.A. and Shahid, M., 2019. Evaluation of lead tolerant plant growth promoting rhizobacteria for plant growth and phytoremediation in lead contamination. *Revista internacional de contaminación ambiental, 35(4), pp.999-1009*.

SAEED, Q., XIUKANG, W., HAIDER, F.U., KUČERIK, J., MUMTAZ, M.Z., HOLATKO, J., NASEEM, M., KINTL, A., EJAZ, M., NAVEED, M. AND BRTNICKY, M., 2021. Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: A comprehensive review of effects and mechanisms. *International Journal of Molecular Sciences, 22(19), p.10529.*

SINGH, D., AGUSTI, A., ANZUETO, A., BARNES, P.J., BOURBEAU, J., CELLI, B.R., CRINER, G.J., FRITH, P., HALPIN, D.M., HAN, M. AND VARELA, M.V.L., 2019. Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease: the GOLD science committee report 2019. *European Respiratory Journal, 53(5).*

STANDFORD, S. AND ENGLISH, L., 1949. Use of flame photometer in rapid soil tests for K and Ca. Agron. J, 41(4), pp.46-4.

Steel, R.G.D. and Torrie, J.H., 1980. Principles and Procedures of Statistics 2nd Edition McGraw-Hill. *New York, New York.*

SUBBAIAH, B.V., 1956. A rapid procedure for estimation of available nitrogen in soil. *Curr. Sci., 25, pp.259-260.*

SUGUMARAN, P. AND JANARTHANAM, B., 2007. Solubilization of potassium containing minerals by bacteria and their effect on plant growth. *World Journal of Agricultural Sciences, 3(3), pp.350-355.*

ZHANG, S., GEDALANGA, P.B. AND MAHENDRA, S., 2016. Biodegradation kinetics of 1, 4-dioxane in chlorinated solvent mixtures. *Environmental science & technology, 50(17), pp.9599-9607.*

ZHAI, Y., ZHU, J.X., TAN, T.M., XU, J.P., SHEN, A.R., YANG, X.B., LI, J.L., ZENG, L.B. AND WEI, L., 2021. Isolation and characterization of antagonistic Paenibacillus polymyxa HX-140 and its biocontrol potential against Fusarium wilt of cucumber seedlings. *BMC microbiology, 21(1), pp.1-12.*