**Isolation and Diagnosis of Paenibacillus sp. from Rhizosphere**  **soil and test its efficiency to solubilize potassium compounds and determine its role in barley growth**

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**Abstract**

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, KSB have been found to dissolve potassium from insoluble K-bearing minerals. In this study, bacterial isolates were obtained from rhizosphere of different plant in one region, 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 used in pot experiment to determine the KSB (biofertilizer) 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 biofertilazer. The values of length plant were highly significant positively correlated with leaf(length, width, area), r= 0. 871\*\*,0.793\*\*, 0.806 \*\* respectively. Both fertilizer had significant influence on water soluble K but only biofertilizer on the available K

**Rhizosphere Bacteria, Potassium Solubilization, Mica Powder, Paenibacillus sp, Sorghum plant**

**Introduction**

Barley(Hordeum vulgare L.) , is a member of the grass family, it is a cereal grain that people can use in bread, beverages, stews, and other dishes, as a whole grain, barley provides fiber, vitamins, and minerals. These offer various health benefits( Dietary Guidelines for Americans, 2010). Increasing the plant growth requires improvement of soil quality especially plant essential nutrients nitrogen, phosphorous and potassium. Potassium is the third most important plant growth-limiting mineral nutrient . It plays an important role in vegetal metabolic processes such as photosynthesis, respiration, macromolecular biosynthesis and disease resistance. In the previous decades, bio-fertilizers have been used in growing crop to supply it with nutrients, stimulate plant growth through the production of plant hormones, inhibit the activity of plant pathogens, improve soil structure (Abdel-Salam and Shams, 2012). Bio-fertilizers are applied to the soil or plant in order to reduce the uses of chemical fertilizers (Archana et al. 2017). Microorganisms play an important role in the nature of the potassium cycle, Some types of rhizosphere bacteria are able to easily dissolve potassium compounds from the soil. Potassium solubilizing bacteria dissolve potassium Specifically aluminum and silicon from insoluble potassium-bearing minerals such as orthoclases, illite and mica By organic acids that dissolve potassium rock or chelated silicon ions to release Potassium in solution(Mahendra etal,.2016), Paenibacillus polymyxa (Formerly called Bacillus polymyxa) is a Gram-positive, spore-forming, rod-shaped bacterium, has many beneficial properties for agricultural(Saeed etal.,2021: Zhai etal.,2021). It is free-living organism that can improve soil health by increasing the availability of nutrients, such as potassium solubilization, and by producing compounds that can enhance soil structure and fertility. Additionally, it can promote plant growth by increasing root development, improving nutrient uptake, and enhancing drought tolerance. Moreover, it act as organ, it is soil conditioner fertilizer to degrade pollutants in grow media, like polycyclic aromatic hydrocarbons and heavy metals Sheng and He,2006). The solubilizing illite and feldspar mediated microorganisms due to the production of organic acids such as acid oxalic, tartaric acid and because of the production of capsular Polysaccharides that help dissolve minerals(Ahmad etal.,2016;Ali etal.,2020), Potassium-solubilizing bacteria affect the movement of potassium in the soil and make it more ready for the plant. From that, we see that the mechanism of analysis of potassium compounds is either by increasing the acidity of the medium or soil due to organic acids produced, and this is what is referred to as Acidolysis or breakdown of insoluble potash compounds by the formation of complex compounds in the medium called Complexolysis , The third mechanism which is called Exchange reaction, It's happening decomposition of insoluble potash compounds through the exchange reactions and this processes are key to the conversion of insoluble potassium compounds that found in minerals into the soluble form of potassium. Which results in increasing the nutrient readiness of the plant and encouraging the chelation of cations (Das,2016), However, continuous and excessive use of chemical fertilizers cause health and environmental hazards, deterioration in soil properties and consequently crop shortages (Etesami etal.,2017). Hence, the current study aimed to isolate and diagnose Paenibacillus sp. from the soil of the rhizosphere for different plants (crops and fruits ) in native soil of Halabja governorate using cultural, microscopic and biochemical methods , test its efficiency in dissolving potassium compounds in the solid and liquid Alexandrov medium and using peanibacillus sp. that isolates as bio-fertilizer in barley growth for explanation their effect.

**Material and methods**

Rizosphere soil samples were collected from15 divers plants(fruits, crops), The studied area is known Tabacora village, sharazoor, which located in the southwestern extension of the Halabja, Kurdistan region of Iraq. This area lies at35.170581 and longitude is 45.974956, for the purpose of isolating bacteria paenibacillus polymyxa, this was done by taking the roots of the plants and the surrounding soil which represents zone zero, samples were also taken from 30 and 50 horizontal distances and 30 and 50 vertical distances as shown in diagram(1).

 **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 placed in sterile plastic bags and then transferred to the laboratory and Kept in the refrigerator until the isolation and diagnosis process was carried out. For the purpose of isolating bacteria dissolving potassium compounds, the Alexandrov medium consisting of 5.0% glucose, 0.05% MgSO4.7H2O, 0.0006% FeCl3, 0.01% CaCO3, 0.2% Ca3PO4 and 0.3% Mica(source of potassium) (Parmar and Sindhu, 2013).media sterilize using autoclave device at 121 C0 temperature and pressure 1.5 pounds inches2 for 20 minutes and medium adjust on 6.5 pH, a serial dilution of soil has been prepared form dilution 10-1 to 10-6 Then 1 ml of dilution (10-6 ) were transferred and by spread method on sterilizer Alexandrov medium that molded in the plates and incubated at (30±1°C) temperature for 7 days , potassium solubilizing bacteria was observed by formation transparent halo around developing colonies on the plates, that indicate the dissolution of the source of insoluble potassium in the medium, purified several times on the plates after which the colonies were selected, and kept in the refrigerator at a temperature of 4 ̊ C until use, The culture properties of bacteria and their response to the color of gram beside the bacteria movement were examined(Atlas etal., 1995).

**Determination of solubilization index (SI)and Potassium Solubilization Efficiency (SE)**

Coefficient of dissolving mineral( mica )estimated as a source of potassium for isolates solubilized potassium, Alexandrov solid medium used for this purpose, the medium was prepared in Petri dishes and left to harden and then transferred 0.1 ml by means of 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 incubated at a temperature of (30±1°C) . After 7 days of incubation measured the diameter of the colonies and the diameter of the transparent halo using the measurement ruler besides with the following equation to estimate the coefficient of dissolution.(1): SI= (Colony diameter + potassium solubilizing ring diameter, mm)/(Colony diameter, mm), (2): Potassium Solubilization Efficiency (SE) = (Solubilization ring diameter, mm) / (colony diameter, mm) × 100.( Mahendra etal.,2016).

**Identification of bacteria**

**Culture tests:**

For the purpose of diagnosing Paenibacillus sp. All the selected isolates were examined for the colony phenotypic characteristics of the developing colonies were observed in terms of their shapes, colors, surface and edges of colonies, the presence of distinctive odors, their transparency and consistency on the nutrient agar (Collee etal.,1996).

**1-** **Motility test:** Test tubes containing semi-solid nutrient agar medium were inoculated with bacteria by stabbing method and after incubation at a temperature of (30±1°C) for 24 hours, the spread of growth outside the stabbing area is an indication of the isolate ability to move (Collee etal.,1996).

**2-Starch hydrolysis :** As described in( Atlas etal.,1995).

**Microscopic tests:**

**1- Gram stain**: used for observing the shapes, arrangement and interaction of the cells with the dye were examined under the lens of the oil microscope strength100 x Zoom (Atlas etal.,1995).

**2- Capsule staining:** This test was done according to(Collee etal.,1996).

**Biochemical tests:**

Included the following : methyl red test, , Oxidase, Voges – Proskauer test, Utilization of citrate as a carbon sources, Urease test, Catalase test, Gelatin hydrolysis, Indole production, starch hydrolysis, Casein hydrolysis and H2S production test were performed.) as reported in( Atlas etal.,1995).

**Quantitative estimation of K released from insoluble K bearing minerals**

The ability of bacterial isolates to release K from broth media (supplemented with 1 per cent muscovite mica). to dissolve potassium in liquid culture medium to which 1% of mica mineral added which was retested transfer of one ml of overnight culture of each isolate was inoculated to 25 ml of Aleksandrov broth (Hu et al., 2006) in three replicates . All the inoculated flasks were incubated for three weeks at (30±1°C). The amount of K released in the broth was estimated at 20 days of incubation from triplicate flasks at each stage in comparison with a set of uninoculated controls. The broth cultures were centrifuged at 6000 rpm for 20 minutes in the centrifuge to separate the supernatant from the cell growth and insoluble potassium. The available K content in the supernatant was determined by flame photometry (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.

**Pot experment**

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. An experiment was conducted in the greenhouse of Salahaddin university, Agriculture Engineering Sciences (gbs kkkkk ) In order to check the effect of KSB on barley crop development. 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 part, 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 C060 min.) to get rid of microorganisms (Elkoca etal.,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):Explain different treatments of pot experiment**

|  |  |  |
| --- | --- | --- |
| No. | treatment | Symbol |
| T1 | B0O0  | Control |
| T2 | B0O1 | Without bacteria + with bitmoss |
| T3 | B1O0  | With (bacteria) Pp+ without bitmoss( Pp:Paenibacillus polymyxa) |
| T4 | B1O1  | With (bacteria) Pp + with bitmoss |

**Soil Physicochemical Parameters**

Pot experiment soil samples were air-dried, crushed, and then sieved using a 2 mm sieve. Physiochemical characteristics were analyzed based on the standard methods described by Burt (Burt ,2004). The soil pH was determined in the soil suspension using a pH meter, ECe was determined 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),Available and soluble soil potassium was extracted by ammonium acetate and measured by the flame photometer (Stanford and English, 1949). Carbonate and bicarbonate used titrimetric method, A hydrometer method used to determine the texture of soils(Burt ,2004),tab. 2 displayed some soil characteristic:

**Table 2. Some physical and chemical properties of the studied soil before planting\*.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Properties**  | **Value** | **Soluble ions Cmol.Kg -1** | **Properties** |  **Value** |
| Bulk density gm.cm-3  | 1.70  | **Cations** |
| ECe ds.m-1  | 0.98  | Ca+2 | 0.318 |
| Soil pH  | 7.85 | Mg+2 | 0.122 |
| SOM gm.kg-1soil | 12.50  | Na+1 | 0.095 |
| CaCO3 gm.kg-1 soil | 265.50  | K+1 | 0.066 |
| Total N. mg.g-1soil | 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 | SO4-2 | 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.

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

**Plant growth parameters**

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

**Statistical analysis**

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

**Result and discussion**

**Isolation and diagnosis of KSB**

Potassium-soluble bacterial isolates were obtained which showing clear zone around develop 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.

**Screening the ability of bacterial isolates to solubilize potassium**

The results showed a variation in the ability of isolates to solubilize potassium mineral( mica), due to the variation of the diameters of the transparent regions around bacterial colonies on the surface of solid culture of Alexandrov media, the diameters range between 4.00-7.82 mm. Through the 15 days of the incubation period. The results showed that isolateKSB-3 achieved the largest solubilization Index7.82mm, while KSB-7 isolate recorded lowest solubilization Index 4.00mm, 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- | KSB1 | 2.10 | 9.90 | 5.71 | 44.07 | 471.43 |
| 2- | KSB2 | 2.10 | 11.90 | 6.67 | 53.50 | 566.67 |
| 3- | KSB3 | 2.30 | 15.70 | 7.82 | 61.00 | 682.61 |
| 4- | KSB4 | 1.80 | 8.80 | 5.88 | 44.84 | 488.43 |
| 5- | KSB5 | 2.00 | 8.30 | 5.15 | 41.88 | 415.00 |
| 6- | KSB6 | 1.70 | 6.30 | 4.71 | 38.22 | 371.13 |
| 7- | KSB7 | 1.50 | 4.50 | 4.00 | 33.00 | 300.00 |
| 8- | KSB8 | 2.20 | 12.10 | 6.50 | 52.80 | 550.82 |
| 9- | KSB9 | 2.00 | 10.50 | 6.25 | 49.50 | 525.00 |
| 10- | KSB10 | 2.00 | 9.00 | 5.50 | 43.77 | 450.00 |

**Determination of the amount of dissolved potassium in broth media**

Further Tests were conducted for the isolates that showed zone of solubilization on Aleksandrov agar medium to estimate their ability to release K from broth media. The results showed that the amount of soluble potassium released from potassium mineral (mica) in the broth by the isolates were studied at 20 days after incubation in lab condition and found in the range of (33.00 to61.00)μg.ml-1. The maximum solubilization was observed in KSB3 which is followed by KSB2 53.50μg.m-1l, KSB8 52.80 μg.ml-1 and KSB9 49.50 μg.ml-1 . 4 isolates showed (44.84,44.07 43.77,41.88) μg.ml-1 KSB4, KSB1, KSB10,KSB5 respectively , while two isolates KSB6,KSB7 gave (38.22,33.00) μg.ml-1 of potassium solubilization from muscovite mica.

Statistical analysis shown highly significant positive correlation between potassium concentration in broth with each of SI,SE,D and d, the values of the correlation coefficient(r) were 0.993\*\*,0.992\*\* between K in broth with SI and SE respectively, as well highly significant positive correlation values between potassium concentration in broth with D and d, r=0.985\*\* and 0.883\*\* in order. The four parameters(SI,SE,D,d) Showed a very good represent potassium concentration in broth hence about bacterial activity, our study showed a positive correlation began with SI and followed by (SE,D, d) as shown from figure 1-(A,B,C,D). Which influenced by the speed of microbial growth and the ability of microbial metabolism, our study was compatible with Chishi (2010) studied the amount of potassium released from muscovite mica in a broth by the ranged were from 2.41µg/ml to 44.64µg/ml. at 20 day and SI value for isolates (SI> 4) was very high, While not agreeing with (Chiang et al, 2013), those who found no correlation between SI value and concentration of potassium solubilizing of broth on all K sources(trachyte, feldspar, leucite). Although some strains of their isolates gave clear zone formation (SI value) when screened on media less than 4 mm, they could optimally solubilize insoluble potassium, on the contrary ,they conclude that the high SI value did not always give high K solubilizing, they concluded that potassium -solubilizing indexes do not always have a correlation with ability to solubilize not-easily soluble potassium sources in liquid medium, the solubilizing index can serve as an indication of the ability of microbes to solubilize not-easily soluble potassium sources, they attributed the reason to the amount of organic acids which produced by isolates is the main indicator for measuring the efficiency of bacteria for dissolving potassium.

 Present study isolates belong to same species and isolated from one soil and environment in one field, the difference lies in the type of vegetation, the variance in the ability of some isolates to dissolve potassium more efficiently than others may be due to plant organic residues, decomposition of organic matter which has a major role in microbial diversification in the strain of one species(Allison et al., 2007)

 The solubilization of K is mainly due to the action of organic acids, both of microbial and plant origin. However, like all physiological processes, K solubilization is directly influenced by external factors such as temperature or pH and nutrient such as sugar type that will determine the efficiency of the process(Verma et al., 2020).

Kaur and Kumar ,2018 found when glucose was replaced with other sugars such as galactose, xylose or arabinose K solubilization was comparatively less among their study isolates, they explained that different plants produce various sugars, which in turn affects the solubility of potassium by bacteria isolates which isolated from different plants.

Brockett and others in 2012 in their study concluded the total microbial community and specific microbial groups were significantly higher in soil under the tree than that of shrub or herb-vine, they attributed the reason to this could be because from herb-vine via shrub to tree, the soil conditions improved and became more suitable for microorganisms, as more nutrient resources were supplied in the latter, actually, the soils under the tree had higher water content and inorganic N content and these properties would be the main reasons for higher soil microbial biomass.

KSB contribute to the release of K+ from K-bearing minerals by several mechanisms, where some isolates can collect all the mechanics for solubilizing K , though some can dissolve potassium in one or two ways. such as released H+ can directly dissolve the mineral K as a result of slow releases of exchangeable K, production the organic and inorganic acids and production of protons the major mechanism of K mineral solubilization, there were also another mechanisms that decomposition of organic materials which produces ammonia and acids such as nitric acid (HNO3) and sulfuric acid (H2SO4). That leads to decreasing soil pH (Meena et al., 2016). Ahmad etal., (2016) they pointed out that KSB are usually present in all soils, although their number, diversity and ability for K solubilization vary depending upon the soil , climatic conditions and their abilities to dissolving K, It has also been known that the type and concentration of the organic acid produced by KSB may be different.



B

A



D

C

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

**Effect of inoculation of Paenibacillus polymyxa and organic fertilizer on growth of Barley plant**

KSB3 isolate we selected among Paenibacillus polymyxa that exhibited maximum solubilizing zone and K concentration in solid and liquid Alexsandrov in this research for studying the effect of bio fertilizer on Barley plants growth, while bitmoss used as organic fertilizer, the pot experiment was conducted in agriculture engineering sciences, in factorial completely randomized design (FCRD)with three replications, the results of pot culture experiment were recorded at 30 days table 1.

**1. 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)cm2 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)cm2,L.n. (4.67) cm, tiller n (2.33)cm. and steam diameter (0.62)cm .

Statistical analysis of the data revealed that there were 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 were 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 L.n with Tiller.n 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 reason to both study fertilizers (bio and organic) supplement the plant with potassium in addition to other basic elements( P.polymyxa can nitrogen fixation and dissolve phosphorus), which lead to movement of water, nutrients and carbohydrates in plant tissue. It's involved with enzyme activation within the plant, which affects protein, starch and adenosine triphosphate (ATP) production. The production of ATP can regulate the rate of photosynthesis, which results in better vegetative growth.

**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 and control group exhibited more leaves, longer primary roots, higher plant height, and higher dry weight, most efficient isolates in present study isolated from Fig plant, may due to different plant residues and organic residues can cause changes in the structure and function of microbial communities in the soil (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 in 2014 used the isolated K-releasing bacteria in watering tobacco plants and compared with the control, found the plant height, leaf length, and total K uptake increased. KSB mechanisms to K-resolving are different, this due to the effect and abilities of KSB to produce different types of organic acids such as (citric, malic, tartaric,formic ). In general, the most important mechanisms known in K mineral solubilization by KSB are (a) by lowering the pH; (b) by enhancing chelation of the cations bound to K; and (c) acidolysis of the surrounding area of microorganism (Meena et al., 2014). Subhashini and Kumar 2014 found potassium nutrients are released slowly from the rock materials and their use as fertilizer often causes insignificant increases in the yield of crops, Therefore, concerted efforts are made to understand the combined effects of rock material addition and inoculation of KSB on nutrient availability in soils and growth of different crops. Many studies have also confirmed that PGPMs increase the growth parameters. Heredia etal., 2019 study found barley inoculated with rod shaped and fluorescent bacterium showed an increase shoot length, root length , fresh weight biomass and potassium content in soil and plant .Saleem etal.,2019 also reported rod shaped and fluorescent bacterium that significantly proven as efficient enhancer of the plant barley has moreover an efficient to solubilize potassium under heavy metals stress conditions. This results compatible with our results where the content of soluble.

**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 | 28.17a | 21.92a | 0.60a | 8.58a | 0.67a | 5.33a | 2.67a |
| B1 | 33.17a | 24.92b | 0.72b | 11.60b | 0.70a | 5.67a | 2.33a |
|  O | O0 | 31.00a | 22.92a | 0.65a | 9.33a | 0.65a | 5.17a | 2.33a |
| O1 | 30.33a | 23.92a | 0.67a | 10.86a | 0.72a | 5.83a | 2.67a |
|  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-1soil** | **Sol.K** **mg.kg-1soil** | **Ex.K** **mg.kg-1soil** |
| B | B0 | 485.15a | 45.37a | 439.78a |
| B1 | 610.10b | 74.08b | 536.03a |
|  O | O0 | 500.05a | 48.41a | 451.65a |
| O1 | 595.20a | 71.05b | 524.15a |
|  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

**Conclusion**

The samples were collected from one location with different plant coverage , the capability of bacteria was variance to solubilize potassium due to plant types, most KSB found in Rhizosphere zone rather than in vertical or horizontal distances from rhizosphere, most important parameter to determine potassium solubilizing is solubilizing index and biofertilizer more efficiency than organic for increasing soluble and avialable potassium content in soils.

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