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Morphological, colonial and biochemical characteristics of k-solubilizing bacterial isolates

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Abstract

The present investigation entitled "Morpho-biochemical characterization and mica-K solubilization capacity of bacterial K-solubilizers *in vitro*" involved a laboratory experiment conducted at Soil Biology Laboratory, Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, U.P. (India). The bacterial isolates were selected based on their morphological and biochemical characteristics. The selected efficient isolates were further examined for their ability to solubilize potassium from insoluble K source. All the isolates were gram +ve, rods, motile. Colony color ranged from hyaline to creamy with smooth margin and elevated colony except isolate KSB 64 which was non-motile and forms rough flat colony. KSB 38 showed its significant superiority over rest of the isolates in forming zone of solubilization.

Keywords: Solubilizing, Bio-inoculants, culture, Mineral, Medium

Introduction

The economic status of developing country like India and its huge investments in importing potassium fertilizers has thrown light on the development of an alternative measure which are more eco-bio friendly. The concentrations of soluble potassium in the soil are usually very low and more than 98% of potassium in the soil exists in the form of insoluble rocks and silicate minerals. Finding the efficient K-solubilizing bacterial isolates to diminish the load on chemical fertilizers would be a strategical measure to overcome this issue. Soil microorganisms influence the availability of soil minerals thus playing a central role in ion cycling and soil fertility (Lian *et al.*, 2010) [9]. Microorganisms in soil are able to solubilize 'unavailable' forms of K-bearing mineral such as micas, illite and orthoclase, by excreting organic acids which either directly dissolves rock K or chelating silicon ions to bring the K into solution (Bennett *et al.* 1998 [2]; Maurya *et al.*, 2014 [10]). Several other microorganisms such as *Aspergillusniger*, *Bacillus extroquens* and *Clostridium pasteurianum* were also found to grow on muscovite, biotite, orthoclase, microcline and micas under *in vitro* condition (Meena *et al.*, 2015) [11]. Most Indian soils are fairly rich in K resources as primary and secondary clay minerals and increasing K availability for plant production. However, available K in some Indian soils is not sufficient for high K-demand crops such as potato, soybean and tomato which may be potentially improved by the application of KSB. In India, very few reports are available on the ability of some bacterial species to solubilize potassium (Datta *et al.* 2010; Bahadur *et al.*, 2016 [4, 8]). However, none of the research works have identified a microorganism suitable as a bio-inoculant for potassium solubilization. Until now, there are no reports in any of the scientific publications about a suitable potassium solubilizing bio-inoculant. At present in India, no recommendations are made by government organizations to the farmers about potassium solubilizing bio-inoculant for the fulfillment of potassium requirement. Thus application of Potassium Solubilizing Bacteria is a promising approach for increasing K availability in soils cultivated for high K demand crops (Meena *et al.* 2016 [11]). However, information on mobilization of K in mined waste mica and their use as K-fertilizer for crop production is still lacking.

Material and Methods

Isolates of Potassium Solubilizing Bacteria (KSB) were obtained from Soil Biology Laboratory, Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, U.P (India). The efficient potassium solubilizing capacity of bacterial isolates were determined. Total 10 bacterial isolates were selected based on their greater potassium solubilizing capacity. Potassium solubilizing bacterial isolates was cultured using Aleksandrov medium (Hu *et al.* 2006) [7] which is a selective medium for isolation of potassium solubilizers.

After seven days fully grown broth culture was used for this study. Aleksandrov's agar plate were prepared and loopful of liquid culture was stricken on agar and incubated at $28\pm 2^{\circ}\text{C}$ for a week. Colony characteristics of all 10 isolates were studied on the basis of their morphological characters such as elevation of colony, color, margin, size of plaque, slime formation, gram reaction and cell morphology. For determination of Zone of solubilization bacterial isolates were inoculated with help of inoculation needle and is stricken on Aleksandrov agar media in petriplates either clockwise or anticlockwise and incubated in BOD for 4, 7 and 10 days. Zone of solubilization can be calculated with the help of scale in cm. The biochemical characterization of the isolates was essentially carried out as per the procedures outlined by Cappuccino and Sherman (1992) [3].

Result and Discussion

Morphological and Colonial characteristics of K-solubilizing bacterial isolates

K-solubilizers were characterized on the basis of their morphology such as color, shape, margin, optical density, elevation and gram reaction (Table 1). All the isolates were found to be gram +ve rods. Rods varied in their length and found that all strains selected in this experimental might be belonging to genus *Bacillus* similarly Gundala *et al.* (2013) [6]. Majority of the isolates were hyaline to creamy in color also given by Diep and Hieu (2013) [5] who also isolated 25 strains on Aleksandrov media and found that colony color appears to be white to creamy in color. Similarly Zarjani *et al.* (2013) [19] gave range of color from yellow to white in six isolates of KSB. The morphological characterization revealed that all potassium solubilizing bacteria were gram positive, rod and motile except KSB 64 was found to be nonmotile. Hu *et al.* (2006) [7] also reported two KSB isolates i.e. KNP413 and KNP414 to be immotile. Most of isolates were found to be highly raised except KSB 13 and KSB 16 which were found to be slightly raised and isolate KSB 64 was found to be flat. On Aleksandrov agar medium, colony formed by most of the isolates appeared to be translucent except colony of KSB 16, KSB 51 and KSB 64 which appeared as opaque.

Zone of solubilization

Isolates that have been tested it shows the solubilization zone which ranged from 0.27 to 4.33cm and KSB 38 recorded highest solubilization zone of 4.33cm at 10 days (fig.1) which was significantly superior to all other isolates similar results were also reported by Rajawat *et al.* (2011) [15] as 1.06 cm to 2.17 cm at 3 days of incubation. Interaction effects of incubation period and isolates were found to be non-significant. Zone of solubilization increases with increase in incubation period. This is in agreement with findings of Prajapati and Modi (2012) [14].

At 10 days of incubation the solubilization zone of KSB isolates was found to be highest which has significant superiority over solubilization zone at 4 days and 7 Days after incubation. Increase in zone of solubilization is due to the action of bacterial isolates by continuous production of organic acids. Likewise solubilization of TCP mediated Aleksandrov agar media result in formation of clear zone around the colony growth area which is referred as "Halo Zone" is due to the continuous release of organic acid by bacterial isolates. Zone of solubilization increased significantly with increase in incubation period. KSB 38 was found to be superior to rest of the isolates.

Solubilization Index

Abilities of the isolated bacteria to solubilize mica was investigated for zone of solubilization index at 4, 7 and 10 days after incubation (fig.2). The solubilization index at 4 DAI ranged from 1.24 to 3.19 cm. Large variation was seen at 4 DAI. The solubilization index at 7 and 10 DAI ranged from 1.98 to 3.26 and 2 to 3.71 respectively. Highest solubilization index was with isolate no. KSB 38 and lowest solubilization index was observed with isolate no. KSB 64. Majority of isolates showed the solubilizing index on Aleksandrov agar medium in the range of 2.21 to 2.46 cm diameter. Interaction effect was found to be significant and final result shows increase in solubilization index with the days of incubation.

Slim production

K-solubilizers produce slime in different amount which are categorizes as low, medium and high slime production. Out of ten, six isolates have greater slime production. Sugumaran and Janarthanam (2007) [18] also reported that K-solubilizing bacteria produce a varying quantity of slime. Slime production can be used as a screening parameter for the determination of better strain. Slime production helps in determining the degradation ability of strain for solubilization of K for insoluble mineral i.e. mined mica. The range of variability seen amongst isolates indicates that it is prudent and necessary to keep the isolation of beneficial bacteria a continuous programme. The additional beneficial traits exhibited by the strains indicate the possibility of isolating a strain with multiple beneficial effects.

Biochemical Characteristics

Starch Hydrolysis Test

All of the bacterial isolates showed significant result for starch hydrolysis test. Isolate KSB 38, KSB 1, KSB 41 and KSB 44 gave fast reaction towards starch hydrolysis (Table 2). Positive starch hydrolysis test with K-solubilizing bacterial isolate is in accordance to the finding of Zarjani *et al.* (2013) [19] who have isolated potassium solubilizers and tested for starch hydrolysis. They reported that all the K-solubilizers isolates have positive starch hydrolysis test.

H₂S Production Test

Result for H₂S was found to be positive for isolate KSB 11, KSB 30, KSB 38 and KSB 44 presented in fig 4.1.15 However 6 isolates among the 10 isolates i.e. KSB 1, KSB 13, KSB 16, KSB 41, KSB 51 and KSB 64 were found to give negative test for H₂S production in 48 hrs (Table 2). Production of H₂S is confirmed by the formation of black coloration in SIM media. Formation of black coloration is due to the sulfide production due to action of bacterial isolate on SIM medium. Observation of this test is similar to findings of Prajapati and Modi (2012) [14]; Meena *et al.* (2015) [12] who have also reported positive H₂S production with K-solubilizers.

Acid Production Test

K-solubilizing bacterial isolates were found to produce different acids which result in conversion of blue color of to green. Table 2 shows the acid production by bacterial isolates. Aleksandrov media when added with BTB remained blue at pH 7 and as the pH dropped color of media changed from blue to green. This indicated production of acids by the K-solubilizers. This result is similar to results of Hu *et al.* (2006) [7]; Prajapati and Modi (2012) [14] who also tested 5 KSB isolates for production of acid. KSB 64 gave negative acid

production test. This might be due to production of very low amount of acid. Sheng and Huang (2002) [17] also found negative acid production test with isolates of K-solubilizers. This indicated that besides production of acids there is other mechanism to solubilize the K from K –minerals by K-solubilizers.

Motility Test

The motile strains alone produced a diffused growth into the medium away from the stab line. Results for the ten isolates were recorded all were found to be motile except KSB 64 which was found to be non-motile. Similar result also reported by Hu *et al.* (2006) [7] in isolate KNP413 and KNP414.

Indole Acetic acid production test

In Indole acetic acid production test, Cherry red coloration on top of Tryptone broth by the isolate KSB 30, KSB 38 and KSB 44 indicated positive indole production test presented in fig 4.1.14 and rest of the isolates shows negative result for the production of IAA. Positive IAA production test also reported by Gundala *et al.* (2013) [6] with strain M9. On the other hand Osman (2009) [13] found negative IAA production with two isolates.

Methyl Red –Voges Proskauer test (MR-VP Test)

Most of the isolates were found to give positive test for the MR-VP test. KSB 1 and KSB 44 were found to give fast reaction. Gundala *et al.* (2013) [6] have also found positive result for M9 strain similarly Prajapati and Modi (2012) [14] found the positive result of three strains KSB 3, KSB 8 and

KSB 11 isolates regarding MR-VP test. Hu *et al.* (2006) [7] also found in two strains about the positive MR-VP test.

Citrate Utilization Test

Out of ten isolates seven isolates gave positive test regarding the utilization of citrate as a principal nutrient source and three isolates i.e. KSB 1, KSB 16 and KSB 64 gave negative test also predicted by Saha *et al.* (2016) [16] performed citrate utilization test on strain M9 which shows negative result and this is in agreement with my experimental finding. Prajapati and Modi (2012) [14] also conducted experiment with five isolates of KSB and found two isolates gave negative test regarding the utilization of citrate (Table 2).

Urease Test

The isolates KSB 1, KSB 11, KSB 16, KSB 30, KSB 38, KSB 41, KSB 51 and KSB 64 showed positive reaction for the degradation of Urea by means of production of enzyme urease. The rest of the two isolates KSB 13 and KSB 44 does not show any change in the coloration of the urea broth thus lacking in its ability to degrade urea (Table 2). Maurya *et al.* (2014) [10] also found that five strains JK3, JK4, JK5, JK6, JK7 showed positive reaction and strain JK2 was found to give negative test. K solubilizers which have ability to indicate the change of color of inoculated broth containing fermentation medium through production of acid. All the isolates fermented glucose effectively. Isolate KSB 1, KSB11, KSB 30, KSB 38 and KSB 51 gave very fast reaction towards conversion of red to yellow in presence of Durham tube. Hu *et al.* (2006) [7] also reported positive carbohydrate fermentation test with K-solubilizers.

Table 1: Morphological Colony characteristics of K-solubilizing bacterial isolates on Aleksandrov agar medium

Isolates	Color	Shape	Gram Reaction	Margin	Elevation		Optical Density		Motility
					Slightly raised	Highly Raised	Translucent	Opaque	
KSB1	Creamy white	Rod	Gram + ve	Smooth elevated	-	+	+	-	Motile
KSB11	Creamy white	Rod	Gram + ve	Smooth elevated	-	+	+	-	Motile
KSB13	Creamy	Rod	Gram + ve	Rough undulated	+	-	+	-	Motile
KSB16	Hyaline	Rod	Gram + ve	Smooth elevated	+	-	-	+	Motile
KSB30	Creamy	Rod	Gram + ve	Smooth elevated	-	+	+	-	Motile
KSB38	Hyaline	Rod	Gram + ve	Smooth elevated	-	+	+	-	Motile
KSB41	Creamy	Rod	Gram + ve	Smooth elevated	-	+	+	-	Motile
KSB44	Hyaline	Rod	Gram + ve	Smooth elevated	-	+	+	-	Motile
KSB51	Hyaline	Rod	Gram + ve	Smooth	-	+	-	+	Motile
KSB64	Creamy	Rod	Gram + ve	Rough flat	-	-	-	+	Non motile

Note: +, positive; -, negative

Table 2: Biochemical characteristics of K-solubilizing bacterial isolates

Isolates Test	KSB 1	KSB 11	KSB 13	KSB 16	KSB 30	KSB 38	KSB 41	KSB 44	KSB 51	KSB 64
Starch Hydrolysis	++	+	+	+	+	++	++	++	+	+
H ₂ S Production	-	+	-	-	+	+	-	+	-	-
Acid Production	++	++	++	++	++	++	++	++	++	+
Motility	++	++	+	+	++	++	++	++	+	-
Indole acetic Production	-	-	-	-	++	+	-	+	-	-
MR-VP	++	+	+	+	+	+	+	++	+	+
Citrate Utilization	-	++	++	-	++	++	+	+	+	-
Urease	+	++	-	++	+	++	+	-	+	+
Carbohydrate Fermentation	++	++	+	+	++	++	+	+	++	+

Note: +, positive; ++ Fast Reaction; -, negative

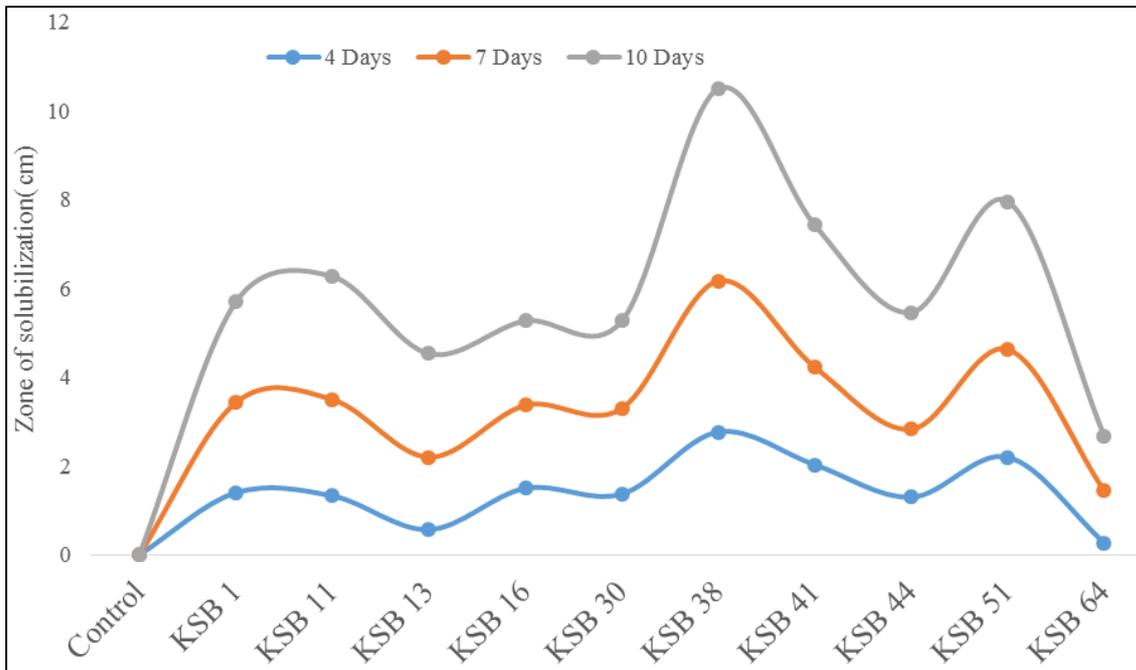


Fig 1: Effect of KSB on Zone of solubilization (cm) of isolates on Aleksandrov agar Medium at different incubation periods

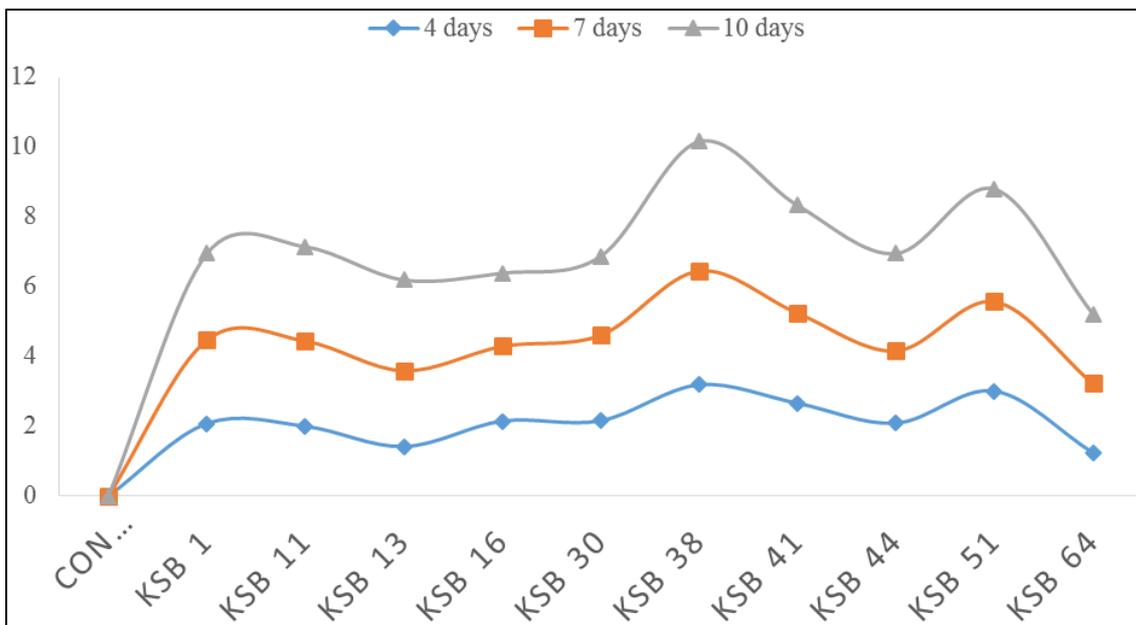


Fig 2: Effect of KSB on Zone of solubilization index at different day's interval

Conclusions

All the isolates were gram +ve, rods, motile. Colony color ranged from hyaline to creamy with smooth margin and elevated colony except isolate KSB 64 which was non-motile and forms rough flat colony. All the isolates of K-solubilizing bacterial isolate produced slime of varying levels which indicated that production of slime may be taken as criteria to screen out the K-solubilizing bacterial isolates. Colony size increased with increase in incubation periods and ranged from 1.19-1.56 cm. KSB 16 showed its significantly superiority over rest of the isolates in developing bigger size of colony. All the bacterial K-isolates showed zone of solubilization on Aleksandrov agar medium and ranged from 0.89-3.50 cm. KSB 38 showed its significant superiority over rest of the isolates in forming zone of solubilization. Zone of Solubilization significantly increases with increase in incubation period and found maximum at 10 days of incubation. All the isolates of bacterial K-solubilizers showed

positive result towards starch hydrolysis, MR-VP test, Acid Production and Carbohydrate Fermentation. Four isolates i.e. KSB 11, KSB 30, KSB 38 and KSB 44 gave positive H₂S production test while six isolates such as KSB 1, 13, 16, 41, 51 and 64 showed negative H₂S production test. Isolate KSB 30, KSB 38 and KSB 44 gave positive test towards indole acetic acid (IAA) production while remaining seven isolate showed negative IAA production test. All the isolates gave positive test towards citrate utilization except KSB 1, KSB 16 and KSB 64 which gave negative test towards utilization of citrate. All the isolates except KSB 13 and KSB 44 showed positive result towards urease production.

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Conflict of interest

The authors did not declare any conflict of interest.

References

1. Barker WW, Welch SA, Chu S, Banfield F. Experimental observations of the effects of bacteria on aluminosilicates weathering. *Amer. Mineral.* 1998; 83:1551-1563.
2. Bennett PC, Choi WJ, Rogers JR. Microbial destruction of feldspars. *Mineral Mag.* 1998; 8(62A):149-150.
3. Cappuccino JG, Sherman N. *Microbiology: A laboratory manual.* The Benjamin/Comings Publishing Company, Inc., California, 1992.
4. Datta SC. Potassium dynamics and status in Indian soil. *Karnataka J. Agric. Sci.* 2010; 24:7-11.
5. Diep CN, Hieu TN. Phosphate and potassium solubilizing bacteria from weathered materials of denatured rock mountain, Ha Tien, Kiên Giang province, Vietnam. *American Journal of Life Sciences.* 2013; 1:88-92.
6. Gundala P, Chinthala P, Sreenivasulu B. A New Facultative Alkaliphilic, Potassium Solubilizing, *Bacillus Spp.* SVUNM9 Isolated From Mica Cores of Nellore District, Andhra Pradesh, India. *J. of Microbiol and Pl. Biotechnol.* 2013; 2(1), 1-7.
7. Hu X, Chen J, Guo J. Two phosphate-and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. *World journal of Microbiology and Biotechnology.* 2006; 22(9):983-990.
8. Indra Bahadur, Bihari Ram Maurya, Ashok Kumar, Vijay Singh Meena, Richa Raghuvanshi. Towards the soil sustainability and potassium-solubilizing microorganisms. Potassium solubilizing microorganisms for Sustainable Agriculture, 2016, 255-266. ISBN: 978-81-322-2774-8.
9. Lian B, Wang B, Pan M, Congqiang L, Henry H. Microbial release of potassium from K-bearing minerals by thermophilic fungus *Aspergillus fumigatus*. *Geochimica et Cosmochimica Acta,* 2010; 72:87-98.
10. Maurya BR, Meena VS, Meena OP. Influence of Inceptisol and Alfisol's Potassium Solubilizing Bacteria (KSB) isolates on release of K from waste mica. *Vegetos.* 2014; 27(1):181-187.
11. Meena VS, Maurya BR, Verma JP. Does a rhizospheric microorganism enhance K⁺ availability in agricultural soils? *Microb. Res.* 2015; 169:337-347.
12. Meena VS, Maurya BR, Verma JP, Aeron A, Kumar A, Kim K, Bajpai VK. Potassium solubilizing rhizobacteria (KSR): isolation, identification, and K-release dynamics from waste mica. *Ecological Engineering.* 2015; 81:340-347.
13. Osman AG. Study of some characteristics of silicate bacteria. *J. S. Tech.* 2009; 13:27-35.
14. Prajapati KB, Modi HA. Isolation and characterization of potassium of potassium solubilising bacteria from ceramic industry soil. *CIB Tech J. of Microbiol.* 2012; 1:2-3.
15. Rajawat MVS, Singh SG, Saxena AK. Isolation and characterization of K-solubilizing bacteria isolated from different rhizospheric soil. In *Proceeding of: Annual Conference of Microbiologists of India.* At Punjab University, Punjab, India, 2011.
16. Saha M, Maurya BR, Meena VS, Bahadur I, Kumar A. Identification and characterization of potassium solubilizing bacteria (KSB) from Indo-Gangetic Plains of India, *Bio. and Agril. Biotech.* 2016; 7:202-209.
17. Sheng XF, Huang WY. Study on the conditions of potassium release by strain NBT of silicate bacteria scientia. *Agricultura- Sinica.* 2002; 35:673-677.
18. Sugumaran P, Janarthanam B. Solubilization of potassium containing minerals by bacteria and their effect on plant growth. *World J. Agric. Sci.* 2007; 3:350-355.
19. Zarjani JK, Aliasgharzag Oustan N, Shahin. Isolation and characterization of potassium solubilizing bacteria in some Iranian soils. *Arch. of Agro. and Soil Sci,* 2013.