INFLUENCE OF POTASSIUM SOLUBILIZING BACTERIA ON GERMINATION AND DEVELOPMENT OF MAIZE (*Zea mays* L.) SEEDLING

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Abstract

Totally five potassium solubilizing bacteria (KSB) were isolated from rhizosphere of grass (*Brachiaria mutica* Staf.) and maize (*Zea mays* L.). To know the density of bacterial inoculum, plate count method was used. To identify the isolated bacteria, basic staining methods and biochemical tests of KB003Hi25^{TM} Identification Kit were used. In order to evaluate the effects of potassium solubilizing bacteria on the growth of maize, five replicates of laboratory experiment with five treatments KSBGR-1(T1), KSBGR-10(T2), KSBMS-1(T3), KSBMR-1(T4), KSBMR-2(T5) and one control were carried out in Microbiology Laboratory, Zoology Department, Pathein University during May 2019 to January, 2020. Seeds were inoculated with solution of 10^8 CFU/mL of KSB and control seeds were not inoculated. The isolated bacterial strains were identified as *Bacillus* sp. The germination percent of maize increase over control at different treatments were 94.4%, 89.6%,85.6%, 82.4%, 78.4%, and 72.8%, respectively in the order T3>T1>T5>T2>T4> Control. In this study, root length and shoot length of treated seedlings increased significantly ($p<0.05$) over control at 4DAS, 6DAS and 7DAS. These results suggested that inoculation of isolated KSB can be considered as efficient alternative biofertilizers to promote maize seed germination and development.

Keywords: Potassium Solubilizing Bacteria, Rhizosphere, biofertilizer, grass, maize

Introduction

Potassium (K) is considered as an essential nutrient and a major constituent within all living cells. Naturally, soils contain K in larger amounts than any other nutrients (Zang and Kong, 2014). Highest proportions of potassium in soils are insoluble rocks and minerals such as micas, illite and feldspar. Potassium involved in the adjustment of plant cellular osmotic pressure and the transportation of compounds in plants. Moreover, potassium promotes the activation of enzymes, the utilization of nitrogen and the syntheses of sugars and protein. Microbes can release soluble K from K-bearing minerals such as K-feldspar, mica and illite.

The use of certain microbes in agricultural soils can assist the solubilization of K in addition to physical and chemical weathering of K minerals (Masood and Bano, 2016). Using K-solubilizing microbes to increase the concentration of available K ions in the soil may mitigate K deficiency (Barker *et al*., 1998). Microbial inoculants that are able to dissolve potassium from minerals and rocks have influence on plant growth and have both economic and environmental advantage (Jabin and Ismail, 2017).

Some bacteria like *Bacillus*, *Thiobacillus*, *Pseudomonas*, *Acidothiobacillus* have been found to simplify and secrete potassium from potassium- bearing minerals in soils (Sheng, 2005 and Liu *et al*., 2012). Some of potassium solubilizing bacteria that capable in dissolving potassium in the soil such as *Paenibacillus* sp., *Bacillus* spp., *B. mucilaginosus* and *B. edaphicus* (Muralikannan, 1996, Sheng, 2005, Sugumaran and Janarthanam, 2017and Liu *et al*., 2012). Berthelin (1983) demonstrated that potassium is solubilized from precipitated forms through production of inorganic and organic acids by *Thiobacillus*, *Clostridium* and *Bacillus*. Microorganisms like *Aspergillus niger*, *Bacillus extroquens*, and *Clostridium pasteurianum* were

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found to grow on muscovite, biotite, orthoclase, microclase and mica in vitro (Archana *et al*., 2013).

Minerals potassium solubilization by microbes which enhances crop growth and yield when applied with a cheaper source of rock potassium may be agronomically more useful and environmentally more feasible than soluble K (Rajan *et al*., 1996). Inoculation of maize and wheat plants with *Bacillus mucilaginosus*, *Azotobacter chroococcum* and *Rhizobium* were used to motilize potassium from waste mica, which in turn acted as a source of potassium for plant growth (Singh *et al*., 2010). Therefore, there are immense possibilities for further increasing the production of crop by application of potassium solubilizing bacteria. Therefore, the present study was conducted with the following objectives are to isolate potassium solubilizing bacteria from the rhizosphere of maize and grass, to investigate the cell characters and staining reactions of isolated bacteria species and to evaluate whether inoculum bacteria could enhance growth parameters of maize.

Materials and Methods

Experimental site and study period

The experiment was conducted at Microbiology Laboratory, Department of Zoology, Pathein University during May, 2019 to January, 2020.

Collection of samples

Maize plants (*Zea mays*) were collected from the cultivated fields of Dawwar Village (17° 2′ 39.08″ N and 95° 27′ 08.06″ E), Pantanaw Township, Ayeyarwady Region and Grasses (*Brachiaria mutica*) were collected from Pathein University Campus (16˚ 48' 19" N and 94˚ 45' 17" E).

Isolation of Potassium Solubilizing Bacteria

One gram of rhizospheric soil or one gram of grinded root pieces was thoroughly mixed with sterilized distilled water and then 10 fold dilution was made. A 20 µL of each dilution was inoculated on Aleksandrov medium (Himedia, India). The plates were incubated at the incubator (30°C) for 7 days and clear zone forming colonies on Aleksandrov medium were selected as potassium solubilizing bacteria (KSBs). Streak plate method was used to purify these selected bacteria. Clear zone formations of isolated bacteria were rechecked by dropping 10µL of bacteria suspension onto Aleksandrov medium.

Biochemical Tests of Isolated Bacteria

Biochemical characters were recorded using KB003 Hi25TM identification Kit (Himedia, India). Selected isolates were added to the wells of kit. Biochemical test characters were recorded and interpretation of the results was following the instructions supplied by the manufacturer. Identification of bacteria was carried out the methods defined in Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Differential Staining Techniques

Gram's staining (Bradshaw, 1992), capsule staining, endospore staining and acid- fast staining (Cruickshank, 1960) were used to identify the bacteria species.

Catalase Test

Catalase test was performed to study the presence of catalase enzyme in bacterial colonies. Fresh culture of pure isolate was taken on glass slide and one drop of hydrogen peroxide was added (Babu *et al*., 2017).

Detection of Motility

Motility of the isolated bacteria can be detected in semi-solid agar medium (Atlas, 2010). Ten milliliter of semi-solid agar was dispensed in test tubes. The tubes containing the medium were inoculated by stabbing with straight wire. After incubation, motile bacteria will spread into the medium and non- motile will confine to the stab.

Enumeration of Bacteria by Standard Plant count method

All isolated bacteria species associated with the rhizosphere of grass and maize were enumerated. The isolated bacteria from slant cultures were placed and grown in peptone water about 24 h and then streaked on Aleksandrov medium plates. After growing, these were inoculated into the test tubes containing 10mL of peptone water. These tubes were incubated at 37˚ C in the incubator for 24 h. Ten fold dilution were then prepared with sterile distilled water and 20µL of each dilution was spread on the surface of plate count agar plates with three replicates. The agar plates were incubated at 37˚C for 24 h.

After incubation, the number of colonies was counted with the aid of colony counter. And the broth culture of viable cell per mL was calculated as suggested by Reynolds and Farinha (2005). Colony forming unit per milliliter or gram of sample=number of colonies/dilution×amount plated

Inoculum preparation

Isolated bacteria, KSBGR -1 (T1), KSBGR-10 (T2), KSBMS-1(T3), KSBMR-1 (T4) and KSBMR-2(T5) were used as inoculums. Bacteria were grown in Aleksandrov's medium. Selected bacteria were grown in 10 mL peptone water for 24 hours at 37ºC. Final concentrations of inoculums were made to 10^8CFU/mL.

Inoculation of seeds

Maize seeds were sterilized with 0.1 % NaOCl for 2 to 3 minutes. The seeds were washed four times with sterilized distilled water. The surface sterilized seeds (25 seeds per each treatment) for treatments were immersed in each inoculum (10^8 CFU/mL) for 3 hrs. Control seeds were only immersed in diluted peptone water without bacteria.

Experimental design

Five treatments and one control with five replications were considered for the experiment: T1= treated with KSBGR-1, T2= treated with KSBGR-10, T3= treated with KSBMS-1, T4= treated with KSBMR-1, T5= treated with KSBMR-2 and Control = without inoculation of bacteria.

Germination condition

Petri dishes with inoculated seeds (25 seeds per dish) were covered with sterilized wet towel and kept in the dark for two days. After two days, seeds were sown in petri dishes containing 1% agar solution (water agar) supplemented with mica source (0.2%). These were kept under light condition at room temperature. Bacterial suspension was added according during the exposure to light.

Germination parameters

One seedling was randomly selected from each petri dish. Measuring of shoot and root lengths were taken from 1 to 7 days after sowing. Percentage of seed germination was calculated by the following equation (Krishnaswami and Sheshu, 1890);

Germination percent (%) = Number of seed germinated / Total number of seeds × 100

Statistical analysis

Data of the experiment were subjected to statistical analysis using IBM-SPSS software (version 25). The differences between the treatment and control means were determine by using One-way ANOVA with LSD, post-hoc test at 0.05 level.

Results

Total five bacterial isolates were selected as potassium solubilizers. Gram staining, acid-fast staining, endospore staining and capsule staining were carried out to identify cell characters (Table 1). Biochemical tests were recorded using KB003 Hi25^{TM} Identification Kit (Himedia, India). All the isolates were Nitrate reduction, Saccharose and Glucose positive, and Phenylalanine Deamination, H_2S production, Voges Proskauer's, Indole, Adonitol, Rhamnose, Raffinose, Trehalose and Oxidase negative (Table 2 and 3). The percentage increase of germination percent have followed the order T_3 (94.4%) > T_1 (89.6%) > T_5 (85.6%) >T_2 (82.4%) > T_4 (78.4%) and control (72.8%) (Table 4). The individual treatment at 1DAS and 2DAS increased over control but the differences are not significant. ANOVA result for root lengths at 3DAS increased significantly while shoot lengths are not significantly different. At 4DAS, root lengths of T2, T3 and T4 treated seedlings were significantly longer than control. Shoot length of all treated seedlings increased over control except T4. At 5DAS root lengths of T2 and T4 inoculated seedlings were significant while shoot lengths of all treatments increased significantly over control at $p<0.05$. At 6DAS, root and shoot lengths of inoculated seedlings were significantly increased over control ($p<0.05$). At 7DAS, root length of T2, T3, T4 and T5, shoot length of all treatments were increased significantly ($p<0.05$) over control (Table 10 and 11).

Table 1 Morphology and Staining reaction of KSB isolates

Isolates	Motility	Cell size (µm)	Cell shape	Arrangement	Gram	Acid-fast	Endospore	Capsule	Catalase
KSBGR-1	+	0.9-1.35	Short rod	Singly/ pair	+	-	+	+	+
KSBGR-10	+	0.9-1.35	Short rod	Singly/ pair	+	-	+	+	+
KSBMS-1	+	0.9-1.35	Short rod	Singly/ pair	+	-	+	+	-
KSBMR-1	+	0.9-1.35	Short rod	Singly/ pair	+	-	+	+	-
KSBMR-2	+	0.9-1.35	Short rod	Singly/ pair	+	-	+	+	-

Table 2 Biochemical Test results of selected isolates (StripI)

No	Strip I	Isolates				
	Test	KSBGR-1	KSBGR-10	KSBMS-1	KSBMR-1	KSBMR-2
1	ONPG	-	-	+	-	-
2	Lysine utilization	-	-	+	+	+
3	Ornithine utilization	-	-	+	+	+
4	Urease	-	-	+	-	+
5	Phenylalanine Deamination	-	-	-	-	-
6	Nitrate reduction	+	+	+	+	+
7	H_2S production	-	-	-	-	-
8	Citrate utilization	-	-	+	+	+
9	Voges Prokauer's	-	-	-	-	_
10	Methyl red	+	+	+	+	+
11	Indole	-	-	-	-	-
12	Malonate utilization	-	-	+	+	+

Table 3 Biochemical Test results of selected isolates (StripII)

No	Strip II	Isolates				
	Test	KSBGR-1	KSBGR-10	KSBMS-1	KSBMR-1	KSBMR-2
1	Esculin hydrolysis	-	+	-	-	-
2	Arabinose	+	+	-	-	-
3	Xylose	+	+	-	-	-
4	Adonitol	-	-	-	-	-
5	Rhamnose	-	-	-	-	-
6	Cellobiose	-	-	+	+	+
7	Melibiose	+	+	-	-	-
8	Saccharose	+	+	+	+	+
9	Ralfinose	-	-	-	-	-
10	Trehalose	-	-	-	-	-
11	Glucose	+	+	+	+	+
12	Lactose	-	-	+	+	+
13	Oxidase	-	_	-	-	-

Table 4 Germination percent of maize at 7 DAS

Treatment	Germination percent (7 DAS)	Percentage of germinated seeds (%)
Control	18.2 ± 2.38^{a}	72.8%
KSBGR-1 (T1)	22.4 ± 2.07^{b}	89.6%
KSBGR-10 (T2)	20.6 ± 2.19^{a}	82.4%
KSBMS-1 (T3)	23.6 ± 1.34^{b}	94.4%
KSBMR-1 (T4)	19.6 ± 2.40^{a}	78.4%
KSBMR-2 (T5)	21.4 ± 2.60^{a}	85.6%

Table 5 ANOVA result for Germination percent (7DAS)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	94.167	5	18.833	3.870	.010
Within Groups	116.800	24	4.867		
Total	210.967	29			

Table 6 Mean Root length of maize plant at 1 to 7days after sowing in control and treatments (n=5)

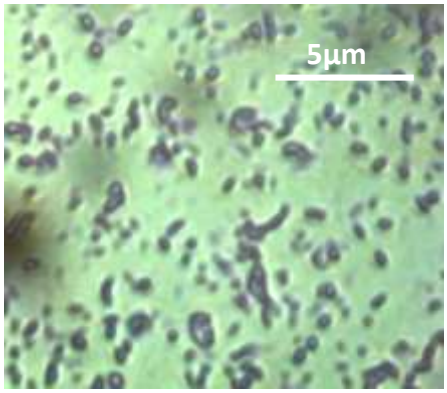
	Root Length (Mean ±SD)						
Treatments	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Control	2.06 ± 0.74[a]	2.92 ± 0.62[a]	1.70 ± 0.31[a]	2.12 ± 0.73[a]	2.00 ±0.51[a]	2.58 ± 0.29[a]	2.80 ± 0.49[a]
T1	3.40 ± 1.42[a]	3.18 ± 1.26[a]	1.92 ± 0.94[a]	2.78 ± 0.77[a]	3.50 ± 1.75[a]	3.74 ± 1.65[a]	4.3 ± 1.65[b]
T2	3.30 ± 1.04[a]	2.64 ± 0.96[a]	2.64 ± 0.47[a]	3.42± 0.58[b]	3.78 ± 0.80[b]	5.56 ±0.87[ab]	4.56 ± 1.09[b]
T3	4.18 ± 1.93[b]	4.30 ± 2.12[a]	3.62 ± 1.43[a]	3.90 ± 0.79[ab]	3.58 ± 2.06[a]	5.18 ± 2.44[b]	5.92 ± 1.92[ab]
T4	4.48± 1.76[b]	4.98 ± 2.33[b]	4.32 ± 2.77[b]	4.54 ± 1.13[ab]	4.06 ± 0.97[b]	4.94 ± 1.49[b]	4.98 ± 1.12[b]
T5	3.36 ± 1.13[a]	3.04 ± 0.97[a]	4.14 ± 1.89[b]	2.62± 0.90[a]	3.58 ± 0.93[a]	4.56 ± 1.20[b]	4.96 ± 1.50[b]

Means followed by a common letter in the same column are not significantly different at 5% level by LSD
T1= KSBGR-1, T2= KSBGR-10, T3= KSBMS-1, T4= KSBMR-1, T5= KSBMR-2, Control= without bacteria

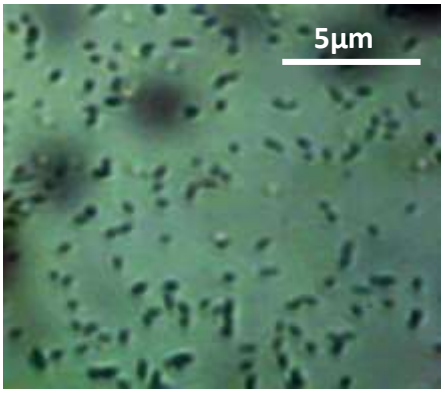
Table 7 Mean Shoot length of maize plant at 1 to 7 days after sowing in control and treatments (n=5)

	Shoot Length (Mean ±SD)						
Treatments	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Control	1.92± 0.39[a]	2.38± 0.33[a]	2.42± 0.34[a]	4.5± 0.49[a]	4.20± 0.36[a]	4.58± 0.62[a]	6.74± 0.78[a]
T1	2.00± 0.54[a]	3.00± 0.72[a]	3.38± 0.95[b]	5.98± 1.02[ab]	7.84± 1.57[ab]	8.76± 1.48[b]	11.54± 1.94[ab]
T2	2.40± 0.65[a]	2.82± 0.66[a]	3.24± 0.92[b]	5.56± 1.08[b]	6.92± 0.96[b]	8.50± 1.33[b]	11.90± 3.75[ab]
T3	2.14± 0.22[a]	2.88± 0.76[a]	3.4± 0.56[b]	5.62± 0.76[ab]	7.68± 1.15[ab]	8.48± 0.76[b]	10.14± 1.73[b]
T4	2.00± 0.50[a]	2.78± 1.03[a]	2.88± 0.28[a]	4.42± 0.41[a]	5.30± 0.90[a]	7.02± 1.10[b]	9.64± 2.40[a]
T5	2.30± 0.76[a]	3.04± 1.49[a]	3.08± 0.29[a]	5.18± 0.46[b]	6.98± 0.58[b]	9.20± 2.71[b]	11.62± 3.35[ab]

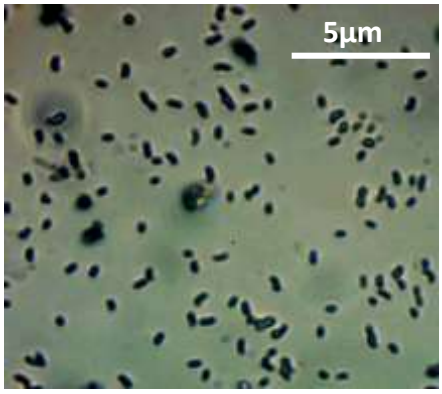
Means followed by a common letter in the same column are not significantly different at 5% level by LSD
T1= KSBGR-1, T2= KSBGR-10, T3= KSBMS-1, T4= KSBMR-1, T5= KSBMR-2, Control= without bacteria



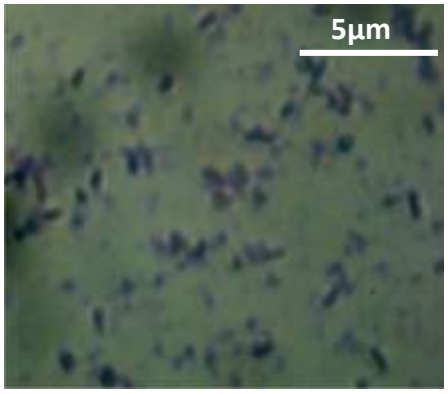
A. Gram Positive Short Rod



B. Not Acid -Fast



C. Endospore

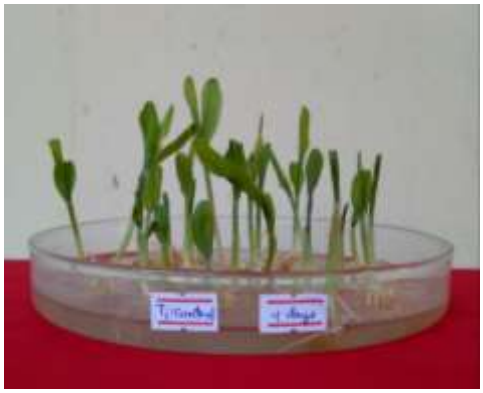


D. Capsulate

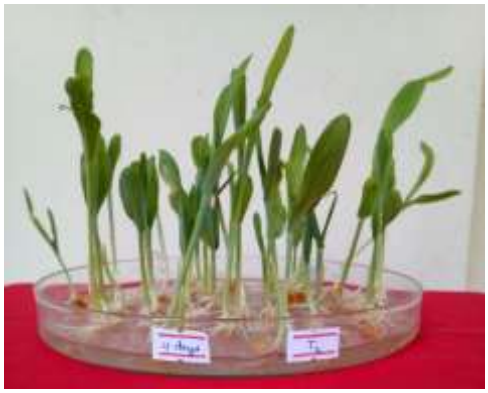
Plate 1 Sample of Staining Reaction of KSBGR-10 (0.9- 1.35 µm)

A. Biochemical Test of KSBGR-10 B. Biochemical Test of KSBMS-1

Plate 2 Sample of Biochemical Test of KSBGR-10 and KSBMS-1



A. Control (4DAS)



B. Treatment (4DAS)

C. Maize Seedlings (1DAS)

D. Maize Seedlings (7DAS)

Plate 3 Sample of Maize Seedlings at 1 to 7days after sowings

Table 8 ANOVA result for Root Length (1 DAS)

	Sum of Squares	**Df**	**Mean Square**	**F**	**Sig.**
Between Groups	17.790	5	3.558	1.816	.148
Within Groups	47.020	24	1.959		
Total	**64.810**	**29**			

Table 9 ANOVA result for Shoot Length (1 DAS)

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	.899	5	.180	.618	.687
Within Groups	6.980	24	.291		
Total	**7.879**	**29**			

Table 10 ANOVA result for Root Length (7 DAS)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26.490	5	5.298	2.978	.031
Within Groups	42.700	24	1.779		
Total	**69.190**	**29**			

Table 11 ANOVA result for Shoot Length (7 DAS)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	94.834	5	18.967	2.967	.032
Within Groups	153.396	24	6.392		
Total	**248.230**	**29**			

Discussion

In the present study isolated strains of KSBGR-1 and KSBGR-10 from the rhizosphere of grass, and KSBMS-1, KSBMR-1 and KSBMR- 2 from the rhizosphere of maize were used as treatments for maize germination. All selected bacterial strains were gram positive, motile, endospore and capsule forming bacteria. KSBGR-1 and KSBGR-10 were catalase positive and KSBMS-1, KSBMR-1 and KSBMR-2 were catalase negative. All the isolates were Nitrate reduction, Methyl red, Saccharose, Glucose positive and Phenylalanine Deamination, H_2S production, Voges Proskauer's, Indole, Adonitol, Rhamnose, Raffinose, Trehalose and Oxidase negative. In Bergey's Manual of Determinative Bacteriology, the characteristics of genus *Bacillus* were rod shape, 0.5-2.5 µm, motile, gram positive, endospore produced, catalase positive and oxidase different reaction in different species (Holt *et al*., 1994).

Prajapati and Modi (2012) expressed that *Bacillus* sp. were Glucose, Arabinose, positive and Urease, Phenylalanine deaminase, Adonitol negative. Parmar *et al*. (2016) stated that *Bacillus* sp. were urea hydrolysis and catalase test positive, and Voges Proskauer and H_2S production negative.

In this investigation, selected isolates were similar with the above observations except in catalase test. Thus, KSBGR-1 and KSBGR-10 may be *Bacillus* species because they were catalase positive as describe in Bergey's Manual. KSBMS-1, KSBMR-1 and KSBMR-2 may be other species because they were catalase negative. Noumavo *et al*. (2013) used 10^8 CFU/mL of rhizobacteria to treat for the growth on maize seed germination and seedling development. In this research, 10^8CFU/mL of KSB was also used to inoculate in the growth experiment of *Zea mays*.

In this research, the percentage of maize germination increased significantly over control ($p<0.05$). The highest germination percent (94.4%) was observed in KSBMS-1 (T3) inoculated seedlings. Noumavo *et al*. (2013) reported that highest germination rate in maize was observed in the treatment with the combination of *Pseudomonas fluorescens* and *P. putida*.

All treatments with potassium solubilizing bacteria (KSB) of this investigation increased significantly ($p<0.05$) over control in root and shoot lengths of maize. In general, seed inoculation with potassium solubilizing bacteria was found to have positive effects on aerial biomass and root biomass in maize plants. This growth promoter effect could be attributed to the potential of these strains to increase the availability of nutrients, such as phosphorus, and siderophore and

phytohormone production (Viruel *et al*., 2014), as well as to their capacity to colonize the root system and interact positively with the plant.

In this observation, some isolated strains were *Bacillus* species and inoculation of these strains with mica resulted the significant effect on the germination percent and growth of maize. The findings by Ahmed (2016) and Sheng (2005) on the maize, cotton and rape respectively corroborated the results obtained this study. They also used the mica and inoculated with potassium solubilizing microorganisms (*Bacillus edaphicus*) to investigate the effect on the root and shoot growth. Similar increase in plant growth parameters due to inoculation of KSB have been reported by several researchers in sudan grass (Basak and Biswas, 2009), and in ground nut (Sugumaran and Janarthanam 2007) when treated with K solubilizing *Bacillus* strains.

Conclusion

The rhizosphere of maize and grass samples were used in the study for isolation of potassium solubilizing bacteria. A total of 5 KSB isolates are isolated on Aleksandrov's medium. All the isolated bacteria were found to be capable of solubilizing K from insoluble K-bearing minerals source. KSBGR-1 and KSBGR-10 may be *Bacillus* species because they were catalase positive as describe in Bergey's Manual while KSBMS-1, KSBMR-1 and KSBMR-2 may be other species. These isolated bacteria were used to examine their influence on the growth of maize seedlings. Currently, the use of chemical fertilizers and manures cannot be refused without avoiding a consequent of abruptly decline in food production. Hence, there is an urgent need for alternative nutrients of plant in agriculture to reduce the adverse environmental effects of chemical fertilizers. The screening method used in laboratory is an available technique to select the effective bacterial strain for the growth and development of particular crop. This study confirms the influence of potassium solubilizing bacteria on germination and development of seedlings. These results suggested the possibility to use these potassium solubilizing bacteria as initial culture of biofertilizer to increase the output of maize.

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