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Growth Promoting Effect of Potassium Solubilizing *Enterobacter hormaechei* (KSB-8) on Cucumber (*Cucumis sativus*) under Hydroponic Conditions

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Abstract

Cucumber requires large amounts of chemical fertilizers which are costly and can be hazardous to the environments when are used excessively. Biofertilizer can play a vital role as substitution to commercially available fertilizer in crop production and reduction of environmental problem to some extent. An experiment was conducted under hydroponics condition using micronutrient containing plant nutrient solution to evaluate the effect of potassium solubilizing bacteria KSB-8 *(Enterobacter hormaechei).* The experiment was a completely randomized design with three replicates. There were four treatments C1 Positive control, C2 Negative Control, T1 : KSB-8 Inoculation with Chemical Fertilizer 25 kg/ha and Rock potassium25 kg/ha and T4: KSB-8 Inoculation with only chemical fertilizer 50 kg/ha. The results indicated that a remarkable increase in root length, flowering fruit setting, fruit maturing, K Content and Chlorophyll content. Thus, it might be concluded that KSB-8 *(Enterobacter hormoechei)* could be used as crop-enhancer and bio-fertilizer for cucumber (*Cucumis sativus*) and other K rich crops under hydroponic conditions.

Keywords: Potassium, *Enterobacter hormoechei*, Hydroponics.

Introduction

Hydroponics is a technology for growing plants in nutrient solutions (water containing fertilizers) with or without the use of an artificial medium (sand, gravel, vermiculite, rockwool, perlite, peatmoss. coir, or sawdust) to provide mechanical support (Douglas, 1975 and Thiyagarajan *et al.*, 2007).Terrestrial plants may be grown with their roots in the mineral nutrient solution only or in an inert medium, such as perlite, gravel, mineral wool, expanded clay or coconut husk. Cucumber (*Cucumis sativus*) is fairly delicate, however, and still require special handling even in a hydroponic system. The impact of nutrients on crops has been evaluated earlier in hydroponics by some authors.

Biofertilizers such as microbial inoculants, which can promote plant growth and productivity, have internationally been accepted as an alternative source of NPK fertilizers. Several reports indicate significant increases in plant growth, total N, and yield of wheat and various grasses following inoculation with N_2-fixing *Azospirillum* species (Boddey and Dobereiner, 1982; Patriquin *et al.* 2011).

Five bacterial isolates evaluated for their ability to reduce root rot caused by *Pythium aphanidermatum* (Pa) on cucumber (*Cucumis sativus* L. *cv. corona*) grown under simulated commercial conditions in a rock wool hydroponic system. Two isolates of *Pseudomonas corrugala* (Pcl3 and Pc35) and three of *Pseudomonas fluorescens* (Pf 15, Pf 16, and Pf27) were evaluated, treatment with Pfl5 alone resulted in significantly greater fruit production compared to plants not inoculated with Pa or treated with biocontrol agents(Rankin and Paulitz, 1994)

Tomatoes and cucumbers could be grown at salinities of 3 to 9 mS /cm either in deep solution culture or NFT, or at four combinations of day and night humidities of 0.1 and 0.8 kPa in rockwool. Increasing salinity had no effect on dry matter accumulation or partitioning in tomato, but reduced the dry weight of cucumber plants and increased the proportion of the total dry weight in the fruit, at the expense of the upper shoot. High salinity reduced Ca uptake mainly in cucumber and, at the same time, increased the proportion of the Ca in the leaves but reduced that in the fruit and upper shoot. In tomato, salinity only increased the proportion in the top but not in the fruit and leaves (Adams and Ho, 1995)

The soilless per litre culture media revealing the optimum dose of Kristalone (18:18:18) fertilizer mixture at 2.5, 2.5, 5.0 and 7.5 g/plant/week can be recommended to obtain maximum yield of cucumber. It is also undoubtedly proved that the pest and disease infestation can be minimized by using perlite as the growing media combined with the application of *Pseudomonas* and neem extract (Kurup *et al.*, 2011).

The study was undertaken in the present investigation, using a hydroponic system to check whether inoculation of cucumber with potassium solubilizing microorganisms (in the presence of insoluble potassium) affects mineral uptake, overall vegetative growth and yield of the plants.

Materials and Methods

A hydroponic cultivation experiment was conducted at Nisharg Farm, Krushicare Consultancy, Lunasan Village, Chhatral, Kadi, (North Gujarat) on growth of Cucumber (*Cucumis sativus*) plant using efficient potassium solubilizing strain: *Enterobacter hormaechei* (KSB-8).

Microorganism

A culture of a potassium solubilizing microorganisms previously isolated from the ceramic industries soil, Bacterial isolate KSB-8 (previously identified as *Enterobacter hormaechei,* using sequence analysis of the small ribosomal subunit, 16S rRNA) is used in this study to determine their influence on growth of cucumber under hydroponic conditions. The bacterium was grown on Nutrient broth medium for 48 hrs on a shaker at 150 rpm and 30^0C.

Plant Material

Cucumber (*Cucumis sativus L.*) seeds variety [NS 499 (KUK 9)] of Namdhari Seeds Pvt. Ltd. Company was used throughout the experiment. Seeds were pre-germinated overnight in 7.5 m*M* Ca $(NO_3)_2$ and planted directly into the hydroponic bed containing coconut peat and inoculums.

Support Medium

One of the most obvious decisions in hydroponic culture is which medium should be used as support medium. Coconut Peat is used as a support medium which is also known as coir. Coconut peat is the leftover material after the fibres have been removed from the outermost shell (bolster) of the coconut. Coir is a 100% natural growth and promoting medium for plants. It is extremely difficult to over water coir due to its perfect air-to-water ratio, water adsorption and percolation, plant roots thrive in this environment, coir has a high cation exchange, meaning it can store unused minerals to be released to the plant as and when it requires it (Kelvin, 2011).

Fertilizer

NPK had positive effect on growth and yield of cucumber as it enhanced cucumber production. Amongst different levels of NPK, application of NPK at 100: 50: 50 kg /ha is the optimum dose for getting maximum production of cucumber per hectare (Jilani *et al*., 2009). P was applied in the form of single super phosphate and N in the form of urea, while potassium was applied in the form of insoluble form of potassium (feldspar) and soluble form of potassium (muriate) according to the treatment schedule.

Hydroponic Experiment

Experiment was conducted for evaluating the effect of potassium solubilizing bacteria (KSB-8) on plant growth and K level of Cucumber plant. Experiment was conducted in the coconut peat (coir) medium containing bed having a 34 meter length, 45 cm width and 30 cm height. Plant nutrient solution used for the

Cucumber plant is 0.24 g of $MgSO_4$, 0.08 g of K_2HPO_4, 0.17 g of K_2 SO_4, 0.344 g of $CaSO_4 \cdot H_2O$, 0.64 g of NH_4NO_3, and 1 ml of the following trace elements (per lit of stock solution): 0.05 g of $FeCl_3$, 0.728 g of KCl, 1.546 g of H_3BO_3, 0.846 g of $MnSO_4 \cdot H_2O$, 0.375g of $ZnSO_4 \cdot 7H_2O$, 0.125 g of $CuSO_4 \cdot 5H_2O$, 0.081 g of H_2MoO_4, 0.001 g of $CoCl_2 \cdot 6H_2O$, pH 7.0 (Yedidia *et al.*, 2001)

Cucumber seeds were surface disinfected in 70% ethanol for 1 min followed by 2.0% NaOCl for 2 min, and thoroughly washed with 2 lit of sterile distilled water. Seeds (20 per box) were put on a sterile gauze sheet, and placed over a sterile stainless-steel screen which held them 1 cm above 300 ml of an autoclaved nutrient solution (Yedidia *et al.*, 2001). Hydroponic experiment was designed for 4 various treatments and 2 control treatments as shown in Table-1. There were four treatments C1 Positive control, C2 Negative Control, T1: KSB-8 Inoculation with Chemical Fertilizern 25 kg/ha and Rock potassium 25 kg/ha and T4: KSB-8 Inoculation with only chemical fertilizer 50 kg/ha. Bacterial inoculum of *Enterobacter hormaechei* (KSB-8) was added to the nutrient solution during the sowing of seeds under hydroponic bed to a final concentration 1 liter containing (10^8 *CFU* /ml) /ha. The different treatments were arranged in a complete randomized block design. The 20 plants sampled for each treatment were considered a random sample. The experiment was carried out for 60 days.

Table-1. Treatments Details for Hydroponic Cultivation of Cucumber

Treatments	Chemical Fertilizer K_2O Kg/ha	Rock potassium (feldspar) K_2O Kg/ha	KSM Application
C1 (Positive Control)	50	-	-
C2 (Negative control)	-	-	-
T1	25	25	KSB-8
T2	50	-	KSB-8

Fertilizer dose : 50 Kg/ha in all the treatments except C2.
T1 and T3 having a K dose of 50% soluble and 50% insoluble K.
T2 and T4 having a K dose of 50 % soluble K_2O.
KSB-8 application : Apply @ 1 liter containing (10^8 *CFU*/ml) /ha at time of sowing.
Irrigation : As and when needed.

Various Plant growth parameters were studied during the growth of the cucumber plant (*Cucumis sativus*) till harvesting i.e. seed germination , Number of Leaves after 2 week of sowing/plant, Number of fruits /plant, Length of Vine seven weeks after sowing, Root Length (cm), Days taken to Flowering, Fruit setting and Fruit maturity etc.

After harvesting the yield parameters of the cucumber is also recorded i.e. fruit length and girth (cm), Fruit weight (gm), yield (tones/hac). Total chlorophyll content (Marker, 1972) and K content (Abul Fadl, 1948) is also measured from the leaves of the cucumber plant.

Results and Discussion

The potassium solubilizing microorganisms showed increasing effect to the growth and yield on the cucumber plant. As shown in the (Table-2) results that the potassium solubilzer having a growth promoting effect in all parameters than control, which gives an indication that this bacteria helped for plant growth and been able to provide the plant with potassium, which is one of the most important nutrients for plant growth, as it promoted rapid growth, increased fruit size and quality, fastened crop maturity, and promoted fruit and seed development.

Table-2 Effects of KSB-8 strains on Growth and Yield Parameters of Cucumber plant. The values are the mean of three replications (± *SD*).

Yield Parameters	Treatments			
	C1	C2	T1*	T2**
Seed Germination	7.0±0.00	7.0±0.0	6.6±0.54	6.4± 0.54
Days taken to Flowering	42.6±2.30	44.2±1.64	**39.8±1.48**	42.4±1.67
Days to fruit setting	19.6±1.51	21±1.22	**17.6±0.54**	18.2±0.83
Days to fruit maturing	12.4±0.54	12.8±0.83	**10.4±1.14**	11.6±0.54
Length of Vine seven weeks after sowing (cm)	99±2.23	93.6±2.50	108.4±2.70	**109.8±4.20**
Root length (cm)	17.6±1.14	15.8±0.83	**19.6±1.67**	19.0±0.70
Number of leaves per plant after 2 week of sowing	3.6±0.54	3.8±0.44	5.2±0.44	**5.4±0.54**
Number of fruits per plant	22.2±1.92	19.4±0.83	23.2±1.09	**23.4±2.30**
Girth of the fruit(cm)	6.26±0.35	5.6±0.35	7.24±0.23	**7.44±0.28**
Length of the fruit(cm)	10.14±0.55	9.6±0.83	**12.44±0.39**	12.34±0.27
Fruit Weight (gm)	95.48±4.77	86.28±3.61	104.54±4.17	**104.94±2.04**
Yield (tons/ hac)	21.84±1.11	20.78±1.08	24.4±0.56	**24.86±2.24**
K content (mg%)	13.86±1.58	10.53±1.95	**17.47±1.76**	16.26±0.40
Total Chlorophyll content (mg%)	1.8±0.3	1.6±0.1	**2.3±0.26**	2.1±0.2

Minerals are an integral part of chlorophyll manufacture through photosynthesis (Mikkelsen, 2008). But lack of other nutrients such as potassium and phosphorus make growth less than the growth of plants with a chemical fertilizer, where potassium is needed for the plant cell's metabolic processes and in influencing the action of enzymes, as well as in aiding the synthesis and translocation of carbohydrates (Ahmad *et al*, 2009).

Potassium is used to build cellulose and aids in photosynthesis by the formation of a chlorophyll precursor. Potassium deficiency may result in higher risk of pathogens, wilting, chlorosis, brown spotting, and higher chances of damage from frost and heat. Potassium (K) concentrations in most plants range from 1 to 4% by weight. Unlike the other primary nutrients, K forms no other compounds in the plant, but remains a lone ion. Potassium is also vital for animal and human nutrition, and thus healthy fruits, vegetables and grains must have adequate levels of K (Brian, 2007).

All the treatments and controls showed seed germination within 6 to 7 days after sowing. The potassium solubilizing microorganisms inoculated seeds showed slightly earlier seed germination compared to the uninoculated controls. This effect could be due to higher IAA and GA production shown by KSB-8. Azcon and Barea (1975) found that strains of *Azotobacter* secreted growth promoting hormones into their culture media. Pre-treatment of seeds with a suspension of *Azotobacter spp.* resulted in improved seed germination and plant growth.

Among the applied treatments treatment 1 i.e. (chemical fertilizer + feldspar +KSB-8) showed increasing effect on the flowering, fruit setting and fruit maturing. Radzki *et al.* (2013) reported bacterial siderophores from C138 are effective in supplying Fe to iron-starved tomato plants when delivered to the roots, independent of the bacterial presence.

Similarly over here the potassium solubilizing microorganisms produce the polysachharides, organic acids and growth promoting substance may be involved in the increased flowering and fruit maturing of the cucumber plant.

The inoculated cucumber plants vine length and root length were more than controls, where root elongation is associated with the production of IAA in early stages. The IAA content was increased in inoculated plants as compared to control and so increased root length, shoot length due to bacterial phytohormones, and the lack of essential nutrient cause the elongation of roots to obtain nutrient (Hamid, 2008 and Hassan *et al*., 2009).

The yield parameters when analysed the highest increased in number of fruits per plant (23.4), Fruit girth (7.44 cm), Length of the fruit (12.44 cm), Fruit weight (104.94 gm),) and Yield (around 25 tons/ ha) were observed in the treatments 1 and 2 which were inoculated with the *E. hormaechei* (KSB-8). It has been reported that, inoculation of corn seeds with *A. brasilense* enhanced the uptake of NO_3^-, $H_2PO_4^-$, and K-ions by root segments (Kapulnik *et al*., 1985). Similar improved nutrient utilization upon inoculation was also observed in sorghum plants (Lin *et al.*, 1983). The highest significant increase in K-uptake by cucumber (17.47 mg %) and Chlorophyll content (2.3 mg%) were obtained in treatment 1. This was associated with dramatic and significant increase in the extent of rhizospheric colonization by these bacteria. As the K-solubilizing activity of KSB-8 is higher which shows an increase in K-uptake, the observed differences in K-uptake might be the result of the ability of KSB-8 to colonize cucumber rhizosphere in high number in the presence of the feldspar rock.

Berthelin *et al.* (1991) reported the stimulation of maize growth by inoculation with phosphate-solubilizing bacteria under hydroponic culture conditions. In this study the potassium solubilizing microorganisms increased the growth and yield of cucumber plant in hydroponic conditions. Belimov *et al*. (1995) studied the effect of inoculation with pure and mixed cultures of nitrogen fixers *Azospirillum lipoferum* 137, *Arthrobacter mysorens* 7 and the phosphate-solubilizing strain *Agrobacterium radiobacter* 10 on growth and mineral nutrition of two barley cultivars and a significant positive effect on grain yield both of the studied barley cultivars was obtained after inoculation. Baset Mia et al. 2010 reported PGPR inoculation significantly increased the root properties (length, volume, mass), shoot growth, the plant height (42–50%), leaf area (128-134%), chlorophyll content (25-33%) and total dry matter of banana plantlets grown under hydroponics condition. Combined effect of the strains *B. thuringiensis* A5-BRSC and *B. megaterium* ATCC 9885 used and proved to accelerate the growth rate of plants (Bandopadhyay, 2015)

Conclusion

Potassium solubilizing bacteria *Enterobacter hormoechei* (KSB-8) have been to exert growth found promoting effects on the cucumber (*Cucumis sativus*) plant under hydroponic conditions. Among the all four treatments the largest increases in plant growth and yield of Cucumber plant are obtained when the *E. hormaechei* (KSB-8) applied as inoculant. Among the two treatments with *E. hormaechei* (KSB-8), the treatment which was applied with the 50%NPK and 50% insoluble potassium rock (feldspar) showed the highest increases in the growth and yield of the cucumber plant. Total chlorophyll, K content and yield of the cucumber plants increased by the application of K solubilizer and reduces the use of chemical fertilizer. K solubilizers can be applied for the mineral solubilization in soluble K deficient soil. This work shows that, under hydroponic conditions, it is possible to enhance the potassium-solubilizing activity by applications of E. *hormaechei* (KSB-8) when an appropriate potassium rock such as feldspar is used. More field tests, particularly under tropical conditions, are required before any recommendation can be made on the beneficial use of *E. hormaechei* (KSB-8) as an inoculant for cucumber fertilized with the feldspar rock potassium.

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