



## Efficiency of Potassium Solubilizing Bacteria Inoculants to Improve Yield of Carrot and Their Potential Cytotoxicity on Root Tip Cells



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**S**TUDYING the ability of potassium solubilizing bacteria (KSB) inoculants to partially replace the conventional potassium mineral fertilizer (KMF) in carrot fertilizing program, in addition to, clarifying their potential cytotoxicity on mitotic chromosomes division of carrot were conducted in the present investigation. According to field and laboratory experiment results, using the potassium solubilizing bacteria (KSB) as biofertilizer positively affected yield and quality of carrot especially, KSB3 inoculate. On the same direction, KSB and KMF combinations were more efficient than those of KSB and KMF alone. Regarding to the cytotoxic studies, data revealed that carrot root tips treated with KSB and their combinations improved cell division and exhibited low cytotoxicity on mitotic division than those treated with KMF alone. So, it could be concluded that potassium solubilizing bacteria (KSB) inoculants could be used alone or mixed with low doses of KMF as alternative of potassium mineral fertilizer (KMF) in carrot fertilizing programs. In addition, it had less cytotoxicity and ecologically safer than KMF.

**Keywords:** Biofertilizer, Organic agriculture, Cytogenetics, Mitotic division, Mitotic index, Mitotic aberrations

### Introduction

Carrot (*Daucus carota* L.) is considered as one of the important root vegetable crops. It is a biennial herb belonging to the family *Apiaceae* with a diploid chromosome number  $2n=2x=18$ . It is often used in salad or in food processing industry due to its high nutritional value (Abo Elkhier, 2013). Its roots are rich in fiber, minerals, calcium, amino acids, vitamin C, glucose and fructose (Bose et al., 2000; Chantaro et al., 2008). In addition, carrot is a good source for  $\beta$ -carotene (vitamin A), vitamin B6 (11% DV) and vitamin K (13% DV) (Soria et al., 2009; Iorizzo et al., 2013). It is easy to grow and does not need much amounts of fertilizers (Allemann and Young, 2002).

In recent decades, the use of chemical fertilizers has been commonly used in crop

production. Besides its high cost, the excessive use of this fertilizers caused numerous hazard effect on environment such as: water pollution, destroyed microorganisms and natural enemies, making the crop more sensitive to diseases and reduced soil fertility as well as, its side effect on human (Aggani, 2013; Wolfe, 2001). This leads to increase the thought of using biofertilizers.

Bio-fertilizers can be referred to as products containing active or latent strains of soil microorganisms, either bacteria alone or in combination with algae or fungi that increase the nutrients availability and plant uptake of mineral nutrients (Vessey, 2003). It give a promising substitute to chemical fertilizers because of it is economical, eco-friendly, more efficient, productive and accessible to small farmers (Gyaneshwar et al., 2002).

Carrot plants need to supply with different plant nutrients. it is a potassium demanding plant (Kadar, 2008). Several studies discussed the role of potassium to increase carrot yield (Anjaiah and Padmaja, 2006; Pekarskas and Bartaseviciene, 2007) and roots quality (Lyngdoh, 2001; Selvi et al., 2005). The majority of K amounts in the soil is unavailable due to imbalanced fertilizer utilization, great increase of crop yield (depleting soil solution K), and the depletion of K in the soil system (Xiao et al., 2017). The use of potassium solubilizing bacteria (KSB) as biofertilizer could be used as a novel approach to convert insoluble form of soil potassium into soluble form.

Plants are good tools for monitoring the toxic effect of various agro-chemicals on different living systems by observing chromosomal aberrations, nuclear DNA amount and micronuclei tests (Singh et al., 2008). The genotoxic and cytotoxic effects of chemical and bio-fertilizers on plants have been demonstrated by many authors (Khaldi et al., 2012, Arora et al., 2014, Bonciu et al., 2018 and Ali et al., 2019).

So, the aim of this investigation is to evaluate the effect of three potassium solubilizing bacteria (KSB) inoculants on yield and quality of carrot plants as well as, their ability to partial replacement of potassium mineral fertilizer (KMF) in carrot fertilizing program. On the other side, to study their potential cytotoxicity on mitotic chromosomes division of carrot plants.

### **Materials and Methods**

Field experiments were carried out at the Ornamental Plants Farm, Faculty of Agriculture, Minia university, El-Minia, Egypt during the two successive seasons of 2016/2017 and 2017/2018 to study the previous mentined aims.

#### *Plant material*

Yellow carrot (*Daucus carota* L.) seeds cv. "Chantenay" were kindly obtained from the Horticultural Research Station, Assuit, Horticultural Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

#### *Inoculant strains*

Three potassium solubilizing bacteria (KSB) namely (Methionineless, Lysineless and Methionineless × Lysineless) (*Streptomyces* spp.) were kindly provided by Genetics Department (Microbial Genetics Lab.) (Dakhly and Uwakiem, 2018), Faculty, Agriculture, Minia Univ., Egypt.

#### *Field experiments*

Seeds of carrot were sown on the 20<sup>th</sup> and 22<sup>nd</sup> October in winter seasons of 2016/2017 and 2017/2018, respectively. The soil texture was clay-loam and physical and chemical properties of the used soil are listed in Table (1).

The experiments were arranged as a split-plot in randomized complete block design with three replicates. The main plots (A) included four levels (0, 25, 50, 75 kg/fed) of potassium sulfate mineral fertilizer (48% K<sub>2</sub>O), while the three treatments of potassium solubilizing bacterial (KSB) inoculants and control (non-treated plants) occupied the sub-plots (B). Therefore, the total of interaction treatments (A×B) were 16 treatments. The sub plot area was 10.5 m<sup>2</sup> (3m wide x 3.5 m long) included 4 ridges, 60 cm apart. Each treatment was separated by one guard ridges. Potassium treatments were added in the form of potassium sulfate (48% K<sub>2</sub>O) at the rate of 25, 50 and 75 kg K<sub>2</sub>O/fed, ammonium sulfate (20.6 % N) at the rate of 60 kg N/fed. and calcium superphosphate (16% P<sub>2</sub>O<sub>5</sub>) at the rate of 40 kg P<sub>2</sub>O<sub>5</sub>/fed.

The first dose of calcium superphosphate was applied during soil preparation while, the second dose was added with the first dose of N and K fertilizers after one month from planting, and the second dose of N and K fertilizers was added after two months from planting. Other agricultural practices were conducted according to the recommendations of the Egyptian Ministry of Agriculture, Agriculture Research Center (ARC). Regarding, biofertilizers treatments, they have been applied as follow:

- Control (without potassium sulphate).
- Zero (potassium sulphate) + KSB1 inoculant (Methionineless) at 5 ml /plant.
- Zero (potassium sulphate) + KSB2 inoculant (Lysineless) at 5 ml /plant.
- Zero (potassium sulphate) + KSB3 inoculant (Methionineless× Lysineless) at 5 ml /plant.
- 25 Kg/fed K (potassium sulphate).
- 25 Kg/fed (potassium sulphate) + KSB1 inoculant (Methionineless) at 5 ml /plant.
- 25 Kg/fed (potassium sulphate) + KSB2 inoculant (Lysineless) at 5 ml /plant.
- 25 Kg/fed (potassium sulphate) + KSB3 inoculant (Methionineless× Lysineless) at 5 ml / plant
- 50 Kg/fed (potassium sulphate).
- 50 Kg/fed (potassium sulphate) + KSB1 inoculant (Methionineless) at 5 ml /plant.
- 50 Kg/fed (potassium sulphate) + KSB2

**TABLE 1. Physical and chemical properties of the used soil at 0-30 cm depth used during the two seasons of 2016/2017 and 2017/2018.**

Constituents	Value	
	1 <sup>st</sup> season	2 <sup>nd</sup> season
Sand (%)	28.20	28.98
Silt (%)	30.70	29.87
Clay (%)	40.10	41.15
Soil type	Clay loam	Clay loam
Organic matter (%)	1.59	1.57
CaCo <sub>3</sub> (%)	2.08	2.10
pH(1:2.5)	7.80	7.77
E.C.(m mhos/cm)	1.06	1.07
Total N (%)	0.07	0.08
Available P (%)	15.15	15.64
Available K <sup>+</sup> (mg/100g)	3.09	3.82
Available Ca <sup>++</sup> (mg/100g)	31.71	31.10
Available Na <sup>+</sup> (mg/100g)	2.42	2.53
<b>Available micronutrients ( EDTA, ppm):</b>		
Fe	8.52	8.22
Cu	2.05	2.02
Zn	2.74	2.85
Mn	8.24	8.09

inoculant (Lysineless) at 5 ml /plant.

- 50 Kg/fed (potassium sulphate) + KSB3 inoculant (Methionineless × Lysineless) at 5 ml /plant.
- 75 Kg/fed (potassium sulphate).
- 75 Kg/fed (potassium sulphate) + KSB1 inoculant (Methionineless) at 5 ml /plant.
- 75 Kg/fed (potassium sulphate) + KSB2 inoculant (Lysineless) at 5 ml /plant.
- 75 Kg/fed (potassium sulphate) + KSB3 inoculant (Methionineless × Lysineless) at 5 ml /plant.

After three months from sowing, samples of 10 plants from each plot were taken to determine N, P and K concentrations in shoots. Shoot samples were separated, and oven dried at 70 °C for 72 h till constant weight, then fine grounded and wet digested. Total nitrogen, phosphorus and potassium contents of roots and shoots were determined according to the methods described by Chapman and Pratt (1961). Chlorophyll in leaves and carotenoids of roots were estimated according to methods of Metzner et al. (1965). At the harvesting time (120 days from sowing), the yield of roots as ton/fed. was calculated. In the same time, samples of 10 plants from each experimental plot were taken to record vegetative

growth parameters (shoot height, number of leaves per plant and shoot fresh weight), yield components (root length, root diameter and root weight), total soluble solids TSS (measured by hand Refractometer) content in root were determined according to methods mentioned by Umiel and Gabelmoii (1971).

#### *Cytological studies*

Cytological studies were carried out at the Laboratory of Cytology, Department of Genetics, Faculty of Agriculture, Minia University, El-Minia, Egypt. Healthy carrot seeds *Daucus carota L.*, were used as biological materials. Four types of experiments were carried out as follow:

- Seeds of carrot were germinated in petri dishes containing cotton wool saturated with distilled water (D.W.) as control.
- Carrot seeds mixed with different KSB (1,2 and 3) inoculates and were germinated as above.
- Another group of seeds were germinated in petri dishes containing cotton wool saturated with different concentration of KMF (K<sub>2</sub>SO<sub>4</sub>) 1500, 2000 and 2500 ppm/L distilled water.
- The last group of seeds were mixed with

different KSB inoculants and were germinated on cotton wool saturated with different KMF concentrations as above.

After 72 hours, roots with 1-2 cm in length for each examined seeds were cut and fixed in freshly prepared farmer's fixative solution (absolute ethyl alcohol: glacial acetic acid, 3:1 v/v) for 24 hours. Fixed roots were kept in 70% ethyl alcohol in the refrigerator until use. Treated roots were washed with distilled water, hydrolyzed in 1 N HCl at 60 °C for 10 minutes then washed by distilled water. The aceto-carmine squash preparation was used for mitotic studies. At least 2000 cells were examined for each treatment (consisting of three seeds). Photographs were taken wherever necessary using Olympus BX51 microscope with a C-4040 Zoom Digital Camera. Mitotic index, phase index and chromosomal aberrations were recorded in each treatment and data were calculated using the formula as described by Racuciu (2009)

Mitotic index (MI) = Number of dividing cells/Total number of observed cells X 100.

Percentage of mitotic chromosomal abnormalities in different stages was counted for Percentage of abnormality = Number of abnormal cells/ Total number of cells X 100.

#### Statistical analysis

The statistical analysis of the field and cytological parameters were carried out using the MSTAT program (Version 4) and means were compared using the Duncans multiple Range Test (DMRT) according to Gomez and Gomez (1984).

## Results and Discussion

### Field experiments

Data of field experiments have been scored after 120 days from seeds sowing, the effect of potassium solubilizing bacteria (KSB) inoculants, different doses of potassium mineral fertilizer (KMF) and their combinations on some vegetative traits in carrot shown in Table (2). The obtained results showed that treatment of carrot plants with KSB3 inoculant alone gave a clear significant increase in plant height trait at the two seasons (53.67 and 53.00, respectively) as compared with all KMF treatments. Almost all KSB and KMF combination treatments showed a significant increase in plant height (PH) trait as compared with all other treatments of the two tested seasons. Treated plants with KSB3+75 Kg/fed KMF gave the highest values PH trait (60.07 and 59.67 cm, respectively) at two successive seasons.

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Plants treated with 75Kg/fed KMF showed insignificant increase in leaves numbers/plant (L. No./plant) trait (15.33) in 1<sup>st</sup> season as compared with all KSB inoculants (15.00, 14.33 and 15.21), while in 2<sup>nd</sup> season the KSB2 and KSB3 inoculants showed insignificant increase in L. No./plant trait (16.00 and 16.10, respectively) as compared with 75 Kg/fed KMF;(15.00) treatment. Treatment with KSB3+75 Kg/fed KMF gave the highest values of L. No./plant at the two successive seasons (19.00 and 18.67, respectively).

Plants treated with KSB2 inoculate showed a significant increase in root length/plant (R.L./plant) trait of the two seasons (16.00 and 16.33cm, respectively) compared with all doses of KMF. KSB1+75Kg/fed KMF which gave the highest values of R.L./plant trait in the two seasons (17.67 and 17.33 cm, respectively). Concerning root diameter/plant (R. Di) trait, plants treated with KSB3 inoculate at the two seasons gave a significant increases in R. Di. (4.30 and 4.40 cm<sup>2</sup> respectively) as compared with all KMF treatments except 75 Kg/fed KMF dose which gave insignificant increase (4.07 and 4.03 cm<sup>2</sup>, respectively). Generally, the results of R. Di. trait of plant treated with all KSB and KMF combinations were better than those of the 75 Kg/fed KMF at the two seasons except those of the KSB1+25 Kg/fed KMF (3.97 cm<sup>2</sup>) and KSB2+25 Kg/fed KMF (3.93 cm<sup>2</sup>) treatments at 2<sup>nd</sup> season.

Treatment of plants with KSB3 inoculate gave a good value in root fresh weight (RFW) values as compared with all doses of KMF treatments. At the two seasons, all KSB and KMF combination treatments gave a high values of RFW as compared with all doses of both KSB and KMF treatments. Treatments KSB1+75 Kg/fed KMF gave the highest values RFW in the two seasons (71.43 and 71.30 g, respectively) .

The obtained results showed that all KSB inoculants treatments increase the values of root dry weight (RDW) in carrot plants, particularly KSB3 which gave high values (8.70 and 8.67g) at the two seasons.

Treatments KSB3+50 Kg/fed KMF and KSB3+75 Kg/fed KMF gave high values of RDW at 1<sup>st</sup> season (9.10 and 9.17, respectively) and (9.07 and 9.03g, respectively) in the 2<sup>nd</sup> season as compared with all other treatments.

Using the 75 Kg/fed KMF dose gave the highest values of leaves fresh weight (LFW) with a significant increase (49.23 and 49.57g) as

compared with all KSB inoculants at the two seasons. On the same side, the KSB3+75 Kg/fed KMF treatments were superior than all other treatments with (52.27 and 51.93g, respectively) during the two seasons. Finally, the data revealed that almost all KSB mixed with KMF treatments gave high increase in root yield (RY) as compared with using KSB or KMF inoculants alone. On the same hand, all KSB inoculants mixed with 75 Kg/fed KMF gave the highest values of RY with a significant increase as compared with all other treatments at two seasons. Mixing all KSB inoculants with the low KMF (25 and 50 Kg/fed) doses gave a considerable increase of RY as compared with using 75 Kg/fed KMF treatment alone at two seasons.

Our finding were similar to the results obtained by Abou El-Nasr and Ibrahim 2011, Abdel Naby et al., 2013, Bakhshandeh et al., 2017 and Xiao et al., 2017) on several vegetable crops. The application of bio-fertilizer supports root growth and this reflect directly on the growth of hole plant (El-Azab and El-Dewiny, 2018). This might be due to their ability to solubilize the organic and inorganic K in the soil (Saha et al., 2016). Improvement of K content of plants can greatly enhance plants' resistance against stress, disease and insect attack (Rehm and Schmitt, 2002). Alternatively, KSB could suppress pathogen attack and increase root growth, and KSB as plant growth promoting rhizobacteria could increase the secretion of bioactive materials and hormones to enhance plant growth (Sattar et al., 2019).

The effect of different KSB inoculants and KMF treatments on N, P and K contents in leaves of carrot were shown in Table (3). Among all KMF treatments, the 75 Kg/fed KMF dose gave the highest values of N, P and K as compared with all other KMF doses at two seasons.

On the same side, the KSB3 inoculant was superior than all other KSB inoculants for increasing of N, B and K contents in leaves of carrot plants at two seasons. Mixing KSB inoculants with low KMF doses (25 and 50 Kg/fed) exhibited considerable increase of N, P and K contents as compared with KMF dose at two seasons.

Plants treated with 75 Kg/fed KMF + KSB inoculants gave the highest values of N, P and K as compared with all other treatments. Concerning the percentages of TSS contents in carrot roots, it was recorded that plant treated

with all KSB inoculants gave a high value of TSS% as compared with all KMF treatments at two seasons. The KSB3 treatment gave the highest value of TSS% as compared with other KSB inoculants (KSB1 and KSB2) at two seasons (7.10 and 7.12, respectively). Plants treated with KSB, KMF combinations showed a clear increase in TSS% as compared with the 75 Kg/fed KMF at two seasons. The highest values of TSS% were found in plant treated with KSB3+75 Kg/fed KMF (8.30) in the 1<sup>st</sup> season, while the treatment KSB3+50 Kg/fed KMF gave the highest at the 2<sup>nd</sup> season (8.27).

The beneficial bacteria, such as potassium solubilizing bacteria (KSB) play an important role in nutrient solubilization, mobilization, mineralization, dissolving, and uptake of nutrients (Meena et al., 2017 and Nath et al., 2017).

Previous investigations have shown that there were significant increase in nutrient uptake by plant as response to inoculations with K-solubilizing microorganisms due to both direct (the ability of solubilizing insoluble silicate compounds minerals in the rhizosphere and plant hormone production) and indirect mechanisms: synthesis of antibiotics, enzymes, fungicidal compounds, and competition with detrimental microorganisms (Bakhshandeh et al., 2015 and Priyanka et al., 2017). Our findings are in agreement with those obtained by Dawa et al. (2012) in sweet pepper, El-Sayed et al., 2018 in Table beet and Roshni et al., 2019 in carrot.

The effect of different KSB inoculants and KMF doses on photosynthesis pigments (Total chlorophyll and carotenoids) in carrot plants were recorded in Table (4).

There were significant increases in the percentages of total chlorophyll in leaves in plants treated with all KSB inoculants except KSB1 which was equal to 75 Kg/fed KMF treatment at the 1<sup>st</sup> season.

Generally, all KSB, KMF combination treatments exhibited a significant increase in total chlorophyll as compared with the treatment (75 Kg/fed KMF) at the both seasons. The treatment KSB2 + 75 Kg/fed KMF gave the highest values of total chlorophyll at the two seasons (1.69 and 1.68 mg/g of FW ,respectively) as compared with all other treatments. On the other hand, the results of total carotenoids of carrot plants treated with KSB inoculants were less than those of the 75 Kg/fed KMF dose except KSB3 inoculant which gave a high value at the two seasons.

**TABLE 2.** Effect of different potassium solubilizing bacteria (KSB) inoculants, potassium mineral fertilizer (KMF) treatments and their combinations on plant height (PH), leaves numbers/plant (L.N.), root length (R.L.), root diameter (R.Di.), root fresh weight (RFW), root dry weight (RDW), leaves fresh weight (RFW) and root yield (RY) of carrot in two seasons of 2016/2017 and 2017/ 2018.

Treatments	P.H. (cm)	L.No.	R.L. (cm)	R. Di. (cm <sup>2</sup> )	R.F.W (g)	R.D.W (g)	L.F.W (g)	RY (Ton)
<b>1<sup>st</sup> season (2016/2017)</b>								
Zero KMF	45.57 J	10.00 H	10.00 H	2.87 H	50.43 H	7.30 F	37.40 G	11.09 H
25 Kg/fed KMF	46.83 IJ	11.33 GH	11.00 GH	3.20 GH	50.90 H	8.03 DE	43.17 F	11.20 H
50 Kg/fed KMF	47.83 I	13.00 G	12.00 FG	3.43 FG	56.23 G	8.30 CDE	46.43 DE	13.10 F
75 Kg/fed KMF	51.27 H	15.33 DEF	14.00 DE	4.07 CDE	59.43 F	8.00 DE	49.23 C	13.18 EF
KSB1	50.93 H	15.00 EF	13.33 EF	3.70 EF	59.57 F	8.20 CDE	44.33 EF	12.37 G
KSB2	50.67 GH	14.33 BCDE	13.21 ABC	4.30 BCD	59.90 EF	8.43 BCD	44.57 E	13.08 F
KSB3	53.67 DEF	15.21 ABCD	16.00 ABCD	3.93 DE	60.07 EF	8.70 ABC	46.53 DE	13.22 EF
KSB1+25 Kg/fed KMF	53.43 FG	14.00 F	14.67 CDE	4.13 CDE	63.30 D	7.73 EF	45.70 E	13.93 D
KSB1+50 Kg/fed KMF	55.23 DEF	17.00 ABCDE	16.67 ABC	4.73 AB	67.00 BC	8.17 CDE	48.43 CD	14.74 BC
KSB1+75 Kg/fed KMF	58.37 ABC	18.00 ABC	17.67 A	4.93 A	71.43 A	9.10 A	49.13 C	15.48 A
KSB2+25 Kg/fed KMF	54.13 EF	15.00 EF	16.00 ABCD	4.10 CDE	62.63 DE	8.07 DE	50.37 ABC	13.78 DE
KSB2+50 Kg/fed KMF	56.23 CDE	15.33 DEF	15.67 ABCD	4.57 ABC	66.20 C	8.67 ABC	49.77 BC	14.56 C
KSB2+75 Kg/fed KMF	56.23 CDE	18.33 AB	16.33 ABC	4.97 A	70.50 A	8.90 AB	51.53 AB	15.51 E
KSB3+25 Kg/fed KMF	56.77 BCD	16.00 CDEF	15.67 ABCD	4.20 CDE	64.70 CD	7.93 DE	49.53 BC	14.24 CD
KSB3+50 Kg/fed KMF	58.63 AB	17.00 ABCDE	17.00 AB	4.73 AB	69.27 AB	9.10 A	50.60 ABC	15.24 AB
KSB3+75 Kg/fed KMF	60.07 A	19.00 A	15.33 BCDE	4.97 A	70.37 A	9.17 A	52.27 A	15.72 A
Mean	53.49FG	15.24EF	14.66CDE	4.18CDE	62.62DE	8.36BCD	47.44DE	13.78DE
<b>2<sup>nd</sup> season (2017/2018)</b>								
Zero KMF	44.23 G	9.00 G	10.33 H	3.20 F	50.10 H	7.07 F	37.07 G	10.92 H
25 Kg/fed KMF	46.50 FG	10.00 G	10.83 GH	3.33 F	51.23 H	8.00 DE	42.83 F	11.17 H
50 Kg/fed KMF	48.50 EF	12.33 F	11.93 FG	3.37 F	55.90 G	8.17 CDE	46.33 E	12.93 F
75 Kg/fed KMF	50.27 E	15.00 DE	14.00 DE	4.03 CDE	59.10 F	8.03 DE	49.57 BCD	12.98 F
KSB1	53.93 D	14.67 DE	13.17 EF	3.57 EF	59.30 F	8.43 BCD	44.67 E	12.19 G
KSB2	49.67 E	16.00 CD	16.00 ABC	4.40 BCD	59.57 F	8.40 BCD	45.67 E	12.89 F
KSB3	53.00 D	16.10 AB	16.33 AB	3.87 E	59.93 EF	8.67 ABC	46.33 E	13.07 EF
KSB1+25 Kg/fed KMF	53.73 D	14.67 EF	14.50 CDE	3.97 DE	63.03 D	7.67 E	49.80 E	13.75 D
KSB1+50 Kg/fed KMF	55.80 CD	16.67 BC	16.33 AB	4.60 AB	66.87 BC	8.13 CDE	48.30 D	14.58 BC
KSB1+75 Kg/fed KMF	58.87 AB	17.67 AB	17.33 A	5.00 A	71.30 A	9.07 A	48.83 CD	15.32 A
KSB2+25 Kg/fed KMF	53.80 D	15.33 CD	15.67 BC	3.93 DE	62.47 DE	8.13 CDE	50.30 ABC	13.62 DE
KSB2+50 Kg/fed KMF	56.13 BCD	15.00 DE	15.33 BCD	4.50 BC	66.03 C	8.63 ABC	51.20 BCD	14.39 C
KSB2+75 Kg/fed KMF	56.13 BCD	18.00 AB	16.00 ABC	4.73 AB	70.30 A	8.83 AB	51.70 AB	15.32 A
KSB3+25 Kg/fed KMF	56.70 BCD	15.67 CD	15.33 BCD	4.07 CDE	64.53CD	7.90 DE	49.47 BCD	14.07 CD
KSB3+50 Kg/fed KMF	58.43 ABC	16.67 BC	16.67 AB	4.63 AB	69.07 AB	9.07 A	50.47 ABC	15.02 AB
KSB3+75 Kg/fed KMF	59.67 A	18.67 A	15.67 BC	5.03 A	70.27 A	9.03A	51.93 A	15.54 A
Mean	53.46D	15.09DE	14.71CDE	4.14CDE	62.44DE	8.33BCD	47.78DE	13.61DE

\*Values followed by the same letters are not significantly different by Duncan's test at 0.05 level.

**TABLE 3. Effect of different potassium solubilizing bacteria (KSB) inoculants, potassium mineral fertilizer (KMF) treatments and their combinations on N, P and K contents of leaves and TSS in roots of carrot at two seasons of 2016/2017 and 2017/ 2018.**

Treatments	N%		P%		K %		TSS%	
	1 <sup>st</sup> season (2016/2017)							
Zero KMF	2.46	D	0.26	DE	2.23	E	5.87	G
25 Kg/fed KMF	2.60	ABC	0.27	BCDE	2.27	D	6.10	FG
50 Kg/fed KMF	2.59	BC	0.28	BC	2.28	CD	6.20	EFG
75Kg/fed KMF	2.62	ABC	0.31	A	2.30	ABC	6.27	EFG
KSB1	2.54	CD	0.29	B	2.29	BCD	6.47	DEFG
KSB2	2.55	CD	0.31	A	2.30	AB	6.27	EFG
KSB3	2.61	ABC	0.31	A	2.31	A	7.10	CD
KSB1+25 Kg/fed KMF	2.59	BC	0.28	BCD	2.28	D	6.20	EFG
KSB1+50 Kg/fed KMF	2.61	ABC	0.29	BC	2.28	D	6.53	DEF
KSB1+75 Kg/fed KMF	2.63	ABC	0.32	A	2.30	ABC	6.73	DE
KSB2+25 Kg/fed KMF	2.57	C	0.23	F	2.27	D	6.70	DEF
KSB2+50 Kg/fed KMF	2.62	ABC	0.26	DE	2.28	D	8.10	AB
KSB2+75 Kg/fed KMF	2.70	AB	0.28	BC	2.30	AB	7.67	B
KSB3+25 Kg/fed KMF	2.62	ABC	0.26	E	2.27	D	7.53	BC
KSB3+50 Kg/fed KMF	2.65	ABC	0.28	BC	2.31	A	8.10	AB
KSB3+75 Kg/fed KMF	2.69	AB	0.27	CDE	2.30	AB	8.30	A
<b>Mean</b>	<b>2.60</b>	<b>ABC</b>	<b>0.28</b>	<b>BC</b>	<b>2.29</b>	<b>AB</b>	<b>6.88</b>	<b>DE</b>
2 <sup>nd</sup> season (2017/2018)								
Zero KMF	2.40	I	0.26	GH	2.23	F	5.67	G
25 Kg/fed KMF	2.60	FG	0.27	FG	2.26	E	6.03	F
50 Kg/fed KMF	2.61	DEF	0.28	EF	2.28	DE	6.13	F
75 Kg/fed KMF	2.62	DE	0.30	BCD	2.30	ABC	6.16	F
KSB1	2.63	CD	0.29	DEF	2.30	AB	6.73	D
KSB2	2.60	FG	0.31	AB	2.28	BCD	6.17	F
KSB3	2.61	DEF	0.31	BC	2.31	A	7.12	C
KSB1+25 Kg/fed KMF	2.59	G	0.28	EF	2.27	DE	6.13	F
KSB1+50 Kg/fed KMF	2.61	EFG	0.29	CDE	2.28	BCD	6.47	E
KSB1+75 Kg/fed KMF	2.68	B	0.33	A	2.30	A	6.57	DE
KSB2+25 Kg/fed KMF	2.57	H	0.23	I	2.27	DE	6.53	DE
KSB2+50 Kg/fed KMF	2.61	DEF	0.26	GH	2.28	CD	8.17	A
KSB2+75 Kg/fed KMF	2.70	A	0.28	EF	2.31	A	7.53	B
KSB3+25 Kg/fed KMF	2.61	DEF	0.25	H	2.26	E	7.37	B
KSB3+50 Kg/fed KMF	2.64	C	0.28	EF	2.31	A	8.27	A
KSB3+75 Kg/fed KMF	2.68	AB	0.29	CDE	2.31	A	8.07	A
<b>Mean</b>	<b>2.61</b>	<b>DEF</b>	<b>0.28</b>	<b>CDE</b>	<b>2.28</b>	<b>CD</b>	<b>6.82</b>	<b>D</b>

\*Values followed by the same letters are not significantly different by Duncan's test at 0.05 level.

Generally, plant treated with all KSB, KMF combination treatments exhibited high or equal values as compared with the 75 Kg/fed KMF dose at the two seasons. Potassium plays key roles to maintain stronger photosynthetic ability with high resistance to photoinhibition and to keep PSII reaction center less damaged and a significantly higher net stomatal conductance in rice (Jia et al., 2008).

Our results are in agreement with those obtained by Panhwar et al. (2011) and Bakhshandeh et al. (2018) whose reported that inoculation of rice seedlings with KSB increase the amount of photosynthesis pigments as compared to noninoculated seedlings. K affects the photosynthesis process at many levels, such as synthesis of ATP, activation of the enzymes involved in photosynthesis, CO<sub>2</sub> uptake, balance of the electric charges required for photophosphorylation in chloroplasts and acting as the counter ion to light-induced H<sup>+</sup> flux across the thylakoid membranes (Marschner, 1995).

#### *Cytological studies*

##### *Phase and mitotic index*

The potential effects of different KSB inoculates, KMF (K<sub>2</sub>SO<sub>4</sub>) doses and their combinations on different phases, mitotic index (MI), different mitotic abnormalities and total mitotic abnormalities (T.Ab) percentages of carrot root tips are shown in Table (5). The obtained results showed that mitotic index MI was significantly decreased with increasing KMF doses in all treated root tips as compared with control (4.71%). On the other hand, treatment with all KSB inoculants exhibited highly significant increase at mitotic index (8.06, 8.46 and 6.46%, respectively).

Carrot root tips treated with 2500ppm KMF gave the lowest value of mitotic index (4.24%) compared with all other treatments including control. Treatment with KMB2 inoculant gave the highest value of mitotic index (8.46%) compared with all other treatments. It was observed that mixing all KSB inoculants with KMF(K<sub>2</sub>SO<sub>4</sub>) doses make increasing in the % MI more than using KMF alone. Concerning the % phases index, the results showed that there were no significant difference between all tested treatments and control at % prophase index except for the combinations of KSB1+1500ppm KMF which gave a lowest value (31.57%). While, root tips treated with KSB1+2500ppm KMF showed the highest value (39.62%) of metaphase index over

than all tested treatments and was significance variance with most other treatments. Regarding the % anaphase index, the highest value was obtained with KSB1+2000ppm KMF(32.74%). Studying the mitotic and phases index could give a clear image about chromosome behavior, segregation and transition of the genetic materials in-between the daughter cells in growing plants. Examine the effect of bio-materials on meristemic cells reflect its mode of action on the mitotic cell division (Ali et al., 2019). Our results were in agreement with those of (Abdel-Hamid, 2007, Verma et al., 2016 and Kyi, et al., 2019).

Using biofertilizers are considered as one of the most important factors to increase crop yield. As mention in previous results biofertilizers significantly increase the percentages of mitotic index, by providing nutrient element which participate in nutrient cycling and benefits crop productivity, stimulating the plant defense response and producing growth regulators like gibberellin and other plant hormones (Montesinos, 2002, Ranjeet et al., 2002 and Singh et al., 2011).

Similar results have been obtained by Tawab et al. (2014) whose reported that biofertilizers Biogene and Potassiomag calibrated by Ssp. of *Streptomyces chiabensis* effect positively on cell division. Chemical compounds such as mineral fertilizer and pesticides caused inhibition of cell division in normal cell lines without any mutation and/or cell changes causes malfunction of a tissue. Their effects are due to, alter protein production, preventing the replacement of cells and thus result in malfunction of the organ where it is located (Gomes et al., 2013a, b). Also, the inhibition of MI it may be due to inhibition of DNA synthesis or blocking of G1 phase, suppressing DNA synthesis or effect of test compound at G2 phase of the cell cycle (Özkara et al., 2015).

##### *Mitotic aberrations*

Many types of chromosomal abnormalities such as; lagging chromosome, chromosomal bridge, chromosome and chromatin fragments, outside chromosome, chromosomal stickiness and micro nuclei have been observed in carrot root tips treated with different KSB, KMF (K<sub>2</sub>SO<sub>4</sub>) and its combinations (Fig.1 and Table 5).

There was a significant increase in total mitotic aberrations in all tested root tips treated with all KMF doses (5.35, 5.69 and 5.79% at 1500, 2000

**TABLE 4. Effect of different potassium solubilizing bacteria (KSB) inoculants, potassium mineral fertilizer (KMF) treatments and their combinations on total chlorophylls in leaves and total carotenoids in roots of carrot at two successive seasons of 2016/2017 and 2017/ 2018 .**

Treatment	Total Chlorophylls in leaves mg/g of FW	Total Carotenoids in roots mg/g of FW
	<b>1<sup>st</sup> season (2016/2017)</b>	
Zero KMF	1.48 H	5.63 B
25 Kg/fed KMF	1.48 H	5.65 B
50 Kg/fed KMF	1.49 H	5.65 B
75 Kg/fed KMF	1.53 G	5.67 B
KSB1	1.53 G	5.66 B
KSB2	1.55 F	5.66 B
KSB3	1.58 DE	5.68 B
KSB1+25 Kg/fed KMF	1.57 E	5.68 B
KSB1+50 Kg/fed KMF	1.59 D	6.35 A
KSB1+75 Kg/fed KMF	1.66 B	5.70 B
KSB2+25 Kg/fed KMF	1.53 G	5.66 B
KSB2+50 Kg/fed KMF	1.67 B	5.68 B
KSB2+75 Kg/fed KMF	1.69 A	5.70 B
KSB3+25 Kg/fed KMF	1.58 DE	5.66 B
KSB3+50 Kg/fed KMF	1.61 C	5.68 B
KSB3+75 Kg/fed KMF	1.66 B	5.69 B
<b>Mean</b>	<b>1.58DE</b>	<b>5.71B</b>
<b>2<sup>nd</sup> season (2017/2018)</b>		
Zero KMF	1.49 H	5.64 B
25 Kg/fed KMF	1.49 H	5.66 B
50 Kg/fed KMF	1.49 H	5.66 B
75 Kg/fed KMF	1.52 G	5.68 B
KSB1	1.53 FG	5.66 B
KSB2	1.55 F	5.66 B
KSB3	1.58 DE	5.71 B
KSB1+25 Kg/fed KMF	1.57 E	5.68 B
KSB1+50 Kg/fed KMF	1.59 D	6.35 A
KSB1+75 Kg/fed KMF	1.64 B	5.70 B
KSB2+25 Kg/fed KMF	1.55 F	5.66 B
KSB2+50 Kg/fed KMF	1.63 B	5.68 B
KSB2+75 Kg/fed KMF	1.68 A	5.70 B
KSB3+25 Kg/fed KMF	1.57 E	5.65 B
KSB3+50 Kg/fed KMF	1.61 C	5.68 B
KSB3+75 Kg/fed KMF	1.65 B	5.70 B
<b>Mean</b>	<b>1.57E</b>	<b>5.72B</b>

\*Values followed by the same letters are not significantly different by Duncan's test at 0.05 level.

**TABLE 5. Effect of different potassium solubilizing bacteria (KSB) inoculants, potassium mineral fertilizer (KMF) treatments and their combinations on % phases, % mitotic index and % different mitotic chromosomal abnormalites in root tips of carrot.**

Treatment	Total no. of examined cells	Total no. of divided cells	%Prophase	%Metaphase	%Ana and Telophase	%MI
Zero KMF	10156	472	50.02 AB	32.03 A	17.95 BC	4.71 CDE
1500 ppm KMF	11195	752	49.82 AB	31.85 A	18.33 BC	6.61 BC
2000 ppm KMF	9158	421	45.97 ABC	32.53 A	21.51 BC	4.59 DE
2500 ppm KMF	9467	393	45.85 ABC	34.73 A	19.42 BC	4.24 E
KSB1	8154	647	41.76 BC	35.42 A	22.82 BC	8.06 AB
KSB2	9394	797	52.25 AB	27.99 A	19.76 BC	8.46 A
KSB3	10680	697	46.34 AB	29.91 A	23.75 B	6.46 BCD
KSB1+1500ppm KMF	6959	397	31.57 C	35.68 A	32.74 A	5.52 CDE
KSB1+2000ppm KMF	8436	429	48.80 AB	28.93 A	22.27 BC	5.05 CDE
KSB1+2500ppm KMF	9195	443	39.93 BC	39.62 A	20.45 BC	4.90 CDE
KSB2+1500ppm KMF	9619	531	45.25 ABC	37.09 A	17.65 BC	5.52 CDE
KSB2+2000ppm KMF	7070	385	57.21 A	27.68 A	15.11 C	5.42 CDE
KSB2+2500ppm KMF	7224	343	39.39 BC	38.65 A	21.97 BC	5.04 CDE
KSB3+1500ppm KMF	10874	635	40.72 BC	38.90 A	20.38 BC	5.85 CDE
KSB3+2000ppm KMF	7752	453	53.44 AB	30.56 A	16.01 BC	5.79 CDE
KSB3+2500ppm KMF	10972	613	45.93 ABC	36.44 A	17.63 BC	5.59 CDE
Mean			45.89 ABC	33.63 A	20.48 BC	5.74 CDE
Treatment	%Laggards	%Bridges	%Fragments	%Outside	%Stickiness	T. Abn%
Zero KMF	0.28 CD	0.40 ABC	0.01B	0.12 B	1.17 CDEF	1.98 DE
1500 ppm KMF	1.08 ABC	0.21 ABC	0.01B	1.05 A	3.01 ABC	5.35 AB
2000 ppm KMF	0.01 D	0.67 ABC	0.01B	1.09 A	3.90 AB	5.69 AB
2500 ppm KMF	0.81 ABCD	0.47 ABC	0.01B	0.77 AB	3.73 AB	5.79 A
KSB1	0.01 D	0.34 ABC	0.01B	0.33 AB	0.67 DEF	1.36 E
KSB2	0.32 BCD	0.16 BC	0.48A	0.34 AB	0.01 F	1.31 E
KSB3	0.27 CD	0.28 ABC	0.01B	0.43 AB	0.65 DEF	1.64 E
KSB1+1500ppm KMF	1.09 ABC	0.14 BC	0.01B	0.81 AB	0.31 EF	2.37 DE
KSB1+2000ppm KMF	0.65 BCD	0.17 BC	0.17AB	0.60 AB	2.23 ABCDE	3.83 BCD
KSB1+2500ppm KMF	0.01 D	0.01 C	0.01B	0.40 AB	4.10 A	4.53 ABC
KSB2+1500ppm KMF	1.21 AB	0.43 ABC	0.01B	0.85 AB	0.30 EF	2.81 CDE
KSB2+2000ppm KMF	0.49 BCD	0.45 ABC	0.01B	0.99 AB	1.23 CDEF	3.17 CDE
KSB2+2500ppm KMF	0.41 BCD	0.93 A	0.01B	0.93 AB	2.09 BCDE	4.36 ABC
KSB3+1500ppm KMF	0.33 BCD	0.79 AB	0.16AB	0.17 AB	0.78 DEF	2.23 DE
KSB3+2000ppm KMF	0.38 BCD	0.33 ABC	0.01B	0.22 AB	2.36 ABCD	3.29 CDE
KSB3+2500ppm KMF	1.62 A	0.23 ABC	0.01B	0.78 AB	1.70 CDEF	4.33 ABC
Mean	0.56BCD	0.38ABC	0.06B	0.62AB	1.77CDEF	3.38CDE

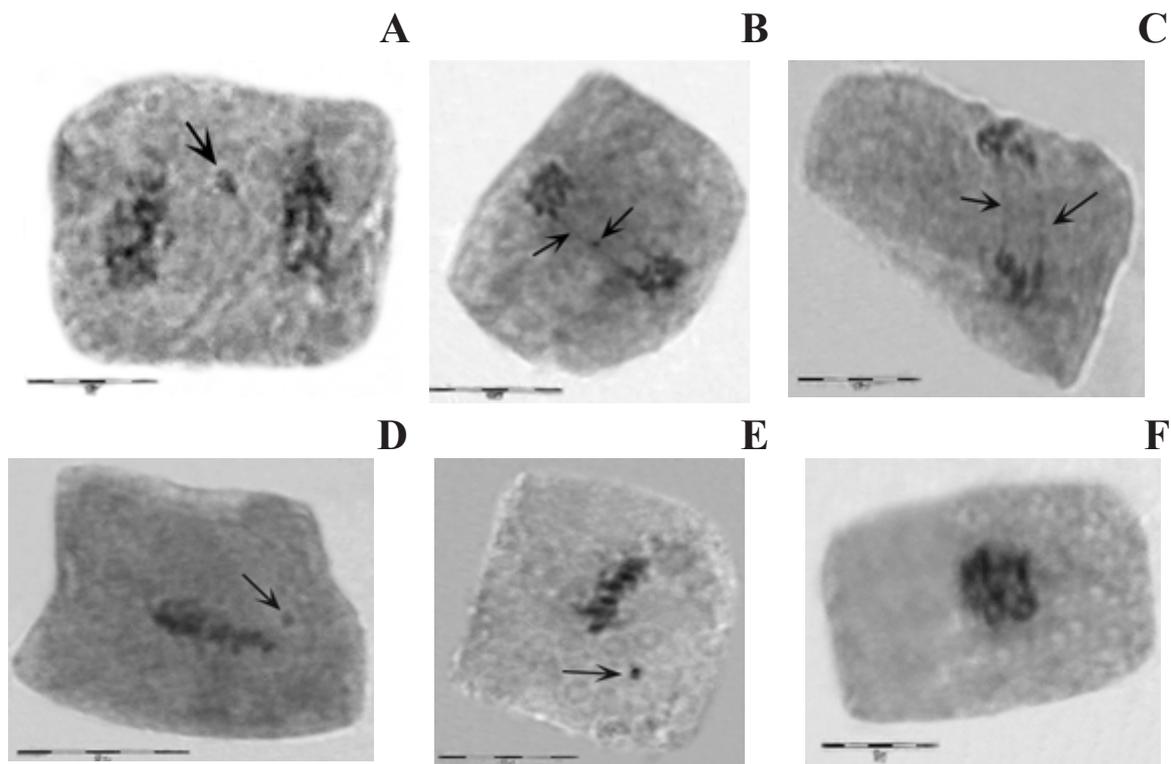
\*Values followed by the same letters are not significantly different by Duncan's test at 0.05 level.

and 2500 ppm, respectively) compared with control (zero KMF) which was 1.98 %. On the other hand, there were insignificant differences in the total mitotic abnormalities in the root tips treated with all KSB inoculants compared with control (1.98%). The highest value of total mitotic abnormalities was recorded in root tips treated with 2500ppm KMF (5.79%) compared with control and all other tested KSB inoculants and their combinations. It was observed that the % T.Ab. obtained by all KSB,KMF combinations were less than these of all KMF doses alone.

Abraham (1997) found that mineral fertilizers produced a significant increase in chromosomal aberrations. Similar observations were also obtained by (Kumar and Naseem, 2011 and Kumar and Gupta, 2008). Stickiness, laggards, bridges, and outside were the most common types of mitotic abnormalities were noticed in this study. The highest value of chromosome lagards were found in root tips treated with KSB3+2500ppm

KMF (1.62%) while the lowest value was in root treated with KSB1, KSB1+2500ppm KMF and 2000ppm KMF with the same value (0.01%) compared with all other treatments. Saxena et al. (2007) reported that laggard could be resulted from inhibition of spindle fibers and this may lead to irregular orientation of chromosomes.

Chromosomal bridges have been seen in all tested root tips. It was found at high frequency in roots treated with KSB2+2500ppm KMF (0.93%) compared with control (0.40%). The formation of bridges might be resulted from chromosomal breakage and reunion (Ata et al., 2008, Osman and Moustafa, 2009). Furthermore, Nassif et al. (2009) attributed bridges and fragments to clastogenic effects which resulted from chromosomal and chromatin breaks. Chromatin fragments were observed in a low frequencies in all tested root tips. Moreover, roots treated with KSB2 gave the highest values of fragments (0.48%) with significant differences as compared with almost



**Fig. 1.** Some types of mitotic chromosomal aberrations at different mitotic stages of yellow carrot (*Daucus carota* L.) root tips treated with different doses of KMF, KSB and their combinations: (A) lagging chromosomes at telophase, (B) bridge and fragment at late anaphase, (C) double bridges at anaphase, (D) fragment at metaphase, (E) outside chromosome at metaphase, (F) chromosomal stickiness at metaphase. Scale bar = 20 microns.

other treatments. Outside chromosome/chromatid have been recorded in a high value in roots treated with all KMF doses (1.05, 1.09 and 0.77%) compared with that of all KSB treatments and control. All KSB2, KMF combinations treatments gave results near to these obtained by KMF alone in the percentages of outside chromosomes.

Chromosome and chromatin stickiness were the most common types of mitotic abnormalities which were noticed in this study. It have been appeared at root tips treated with all tested treatments. Treatment with all KMF doses gave the highest values of stickiness (3.01, 3.90 and 3.73%) compared with all KSB treatments and control. All KSB inoculants and their interaction with KMF doses gave a low frequencies of stickiness except KSB1+2500ppm KMF which gave the highest significant value (4.10%) compared with all KSB and control treatments. Stickiness might be caused by folding of the chromosomes together and the chromosomes become compacted to each other by sub chromatid bridges. Chromosome stickiness leads to inactivation of DNA replication, increased chromosomal contraction and condensation or nucleoproteins probably leading to cell death (Khanna and Sharma, 2013, Osman et al., 2007 and Klasterska et al., 1976). The obtained results revealed that chromosomal aberrations were present in both cases but, more pronounced in the case of KMF. Abraham (1997) found that mineral fertilizers produced a significant increase in chromosomal aberration. Similar observations were also obtained by Kumar and Naseem (2011), Kumar and Gupta (2008). Oney and Tabur (2013) concluded that  $K_2SO_4$  may be has a harmful effect on mitotic chromosomes of *Vici faba* unless are used by suitable doses.

### Conclusion

Field studies showed that using the potassium solubilizing bacteria (KSB) as biofertilizer affected positively yield and quality of carrot plants. On the same direction, KSB and KMF combinations were more efficient than those of KMF doses alone. Regarding to cytotoxic studies, data revealed that carrot root tips treated with KSB and their combinations improved cell division and exhibited low cytotoxicity on mitotic division than those treated with KMF alone. So, it could be concluded that potassium solubilizing bacteria (KSB) inoculants could be used alone

or mixed with low doses of KMF as alternative tools of potassium mineral fertilizer (KMF) in carrot fertilizing programs. As well as, it had less cytotoxicity and ecologically safer than KMF alone.

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### Conflict of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication

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## كفاءة اللقاحات البكتيرية المذيبة للبوئاسيوم على تحسين انتاجية الجزر وتقدير مدى سميتها المحتملة على خلايا القمم النامية

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اجريت دراسة لقدرة ثلاثة لقاحات جديدة من البكتريا المذيبة للبوئاسيوم (KSB) على الاستبدال الجزئي للسماد المعدني البوئاسي (KMF) في برنامج تسميد الجزر ، بالإضافة إلى دراسة السمية الخلوية المحتملة لها على الكروموسومات الميتوزية للجزر. أظهرت النتائج المتحصل عليها من تجارب الحقل والمعمل ان استخدام البكتيريا المذيبة للبوئاسيوم (KSB) كسماد حيوي أثرت بشكل إيجابي على صفات نمو وجودة محصول الجزر خاصة تلك ، المعامله بلقاح KSB3. وفي نفس الاتجاه ، كان استخدام خلطات من البكتريا المذيبة للبوئاسيوم KSB مع جرعات السماد البوئاسي المعدني KMF أكثر كفاءة من تلك الخاصة بكلاهما على حده . فيما يتعلق بدراسة السمية الخلوية ، أظهرت النتائج أن خلايا القمم النامية لجذور نبات الجزر المعالجة بالبكتريا المذيبة للبوئاسيوم KSB وكذلك المعامله بخلطات منها مع التسميد المعدني العادي وجود تحسين في انقسام الخلايا وسمية خلوية منخفضة على الكروموسومات الميتوزية عن تلك التي عوملت باستخدام التسميد البوئاسي المعدني KMF بمفرده. لذلك ، يمكن إستنتاج أن استخدام القاحات البكتيرية المذيبة للبوئاسيوم (KSB) بمفردها أو بعد خلطها بجرعات منخفضة من التسميد البوئاسي المعدني العادي KMF يمكن استخدامها كإداه بديلة لسماد البوئاسي المعدني (KMF) في برامج تسميد الجزر . بالإضافة إلى ذلك ، كان لديها سمية خلوية أقل وأكثر أمانًا من الناحية البيئية من التسميد البوئاسي المعدني KMF.