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IMPROVING MINERAL UPTAKE AND PRODUCTIVITY OF SUPERIOR GRAPEVINES USING SOME STRAINS OF SOLUBILIZING POTASSIUM BACTERIA

Omar F. Dakhly[1*] and M.Kh. Uwakiem[2]

1. Genet. Dept., Fac. Agric., Minia Univ., Egypt

2. Viticulture Res. Dept., Hort. Res. Inst., ARC, Giza, Egypt

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ABSTRACT: A laboratory experiment was conducted during 2015 and 2016 seasons to improve uptake and productivity of grapevines by producing three newly strains of solubilizing K bacteria (Histidineless, Argnineless and Phenylalanineless) by exposing the wild *Streptomyces sp*. with different doses of Ultraviolet irradiation from one to five minutes. These newly K strains as well as the slow release K fertilizer feldspar was used as a partial replacement of mineral K namely potassium sulphate (48% K_2O). Supplying the vines with K as 50 to 75% mineral K plus feldspar as a slow release K fertilizer at 25 to 50% and any one of the three K bacterial strain each at 5 to 10 ml had an announced promotion on growth characteristics, pigments, N, P, K, Mg, Ca and S, berry setting (%), yield as well as physical and chemical characteristics of the berries relative to the application of K as mineral K alone. The best K strain was Histidineless followed by Phenylalanineless and strain Argnineless occupied the last position. The berries quality was remarkably enhanced due to supplying the vines with K as 50% mineral K + feldspar at 50% + Argnineless strain at 10 ml/vine. For promoting yield of superior grapevines, it is advised to supply the vines with K as 50% inorganic K plus 50% feldspar and Histidineless strain at 10 ml/vine. Supplying the vines with K as 25% mineral K + 75% feldspar + 20 ml (Argnineless strain) gave the best results with regard to berries quality.

Key words: Mineral K, K bacterial strains, feldspar, yield, berries quality, superior grapevines.

INTRODUCTION

Recently, many attempts were accomplished for enhancing the efficiency of exportation of superior grapes to foreign markets by using bio organic sources of fertilizers as partially alternatives to chemical ones. The application of organic minerals enriched with silicate bacteria is responsible for stimulating growth and yield of horticultural crops **(Sindhu *et al*., 2012)**. The enhancement of plant K nutrition might be due to the stimulation of root growth or the elongation of root hairs by specific microorganisms (**Mengel *et al*., 2001**). Therefore, the K-solubilizing bacteria are extensively used as biofertilizers. Available K is considered to be a major limiting factor to feed production in many agricultural soils. The application of K-solubilizing bacteria as biofertilizers for agriculture improvement can reduce the use of agrochemicals and support ecofriendly crop production (**Archana *et al*., 2013**). Therefore, it is imperative to isolate more species of mineral-solubilizing bacteria to enrich the pool of microbial species and genes as microbial fertilizers, which will be of great benefit to the ecological development of agriculture (**Liu *et al.,* 2012**). Soil-plant-microbial interaction has got much important in recent decades. Many types of microorganisms are known to inhabit soil, especially rhizosphere

*** Corresponding author: Tel. :** +201001932127
E-mail address: faissalfadel@yahoo.com

and play an important role in plant growth and development (**Yagodin, 1990**). There are abundant microorganisms thriving soil, especially in the rhizosphere (**Mengel *et al.,* 2001**). They are able to easily multiply in a rhizosphere to promote plant growth and yield (**Vessey, 2003**). K-solubilizing bacteria are able to release potassium from insoluble minerals (**Zeng *et al.,* 2012; Gundala *et al*., 2013; Zhang *et al*., 2013)**. In addition, researchers have discovered that K-solubilizing bacteria can provide beneficial effects on plant growth through suppressing pathogens and improving soil nutrients and structure. These bacteria are widely used in biological K-fertilizers and biological leaching **(Lian *et al*., 2002)**.

A wide range of bacteria namely *Pseudomonas*, *Burkholderia,* Acidothiobacillus *ferroxidanse Bacillus mucilaginosus*, *Bacillus edaphicus*, *Bacillus circulans*, *and Paenibacillus* spp have been reported to release potassium in accessible form from K-bearing minerals in soils (**Liu *et al*., 2012)**. Inoculation with the KSMs have been reported to exert beneficial effects on growth of pepper and cucumber (**Han *et al*., 2006**). Similary, inoculation with *Bacillus mucilaginosus, Azotobacter chroococcum*, and *Rhizobium* resulted in significant higher mobilization of potassium from waste mica, which in turn acted as a source of potassium for plant growth (**Singh *et al*., 2010**). Currently, little information is available on the K-solubilization by bacteria, their mechanisms of the KSB inoculation on nutrient availability in soils and growth of different crops. **Sheng and Huang (2002)** found that K release from the minerals was affected by pH, oxygen, and the bacterial strains used. The efficiency of the K-solubilization by different bacteria was found to vary with the nature of potassium bearing minerals and aerobic conditions. The extent of potassium solubilization by *B. edaphicus* in the liquid media was more better growth was observed on illite than feldspar **(Sheng and He, 2006)**.

Kannaiyan (2002) summarized the merits of using K biofertilizers besides K slow release fertilizers for enhancing the release of K as well as promoting yield and berries quality of Thompson seedless grapevines. **Shaaban (2014)** pointed out that amending Superior grapevines with mineral N at 50 K and 50% plant compost with feldspar and the K biofertilizers potassium was accompanied with enhancing growth, vine nutritional status, yield and quality of the berries compared with those vines received N as 100 mineral N. Previous studies showed that using K biofertilizers besides mineral, slow or fast release fertilizers had an announced promotion on fruiting of fruit crops **(Yagodin, 1990; Sheng and Huang, 2002; Ahmed and Abada, 2012; Shaheen *et al*., 2013; Allam, 2014; Shaaban, 2014; Ahmed *et al*., 2016)**.

The target of this study was examining the effect of using the slow release K (feldspar) and some K microbial strains (Histidineless, Argnineless and Phenylalanineless) as a partial replacement of inorganic K fertilizer on growth and fruiting of Superiro grapevines.

MATERIALS AND METHODS

Lab studies were carried out in Genetics Dept., Fac., Agric., Minia Univ., Egypt while the field experiment was done in located at El-Hawarta Village, Minia District, Minia Governorate, Egypt. These studies have proved new isolates of *Streptomyces* and their effects on yield and its components of Superior grapevines under Minia Governorate conditions.

Strains

Streptomyces, spp. was kindly provided by Genetics Department (Microbial Genetics Lab.), Fac., Agric., Minia Univ., Egypt.

Media

Complete medium (CM) **(Dulaney, 1951)** and minimal medium (MM) **(Chesters and Rolinson, 1951)**.

Indication, Isolation and Identification of Auxotrophic Mutants by Ultraviolet (UV)

Samples of 0.1 ml *Streptomyces* wild type spread on plates and treated with different times (1, 2, 3, 4 and 5 minutes) to ultraviolet exposure. The plates were incubated at 30^{o}C for four days and the surviving colonies were tested on MM and CM media and incubation 5 days at 30^{o}C. The survival percentages were scored by counting the growing cells.

$$\text{Survival percentages} = \frac{E}{C} \times 100$$

Where:

E= total number of grown cells in each treatment

C= total number of grown cells in the control.

Identification of *Streptomyces* spp. auxotrophic mutants were carried out according to **Holiday (1956)**.

Standard inoculum

It was carried out by the propagation of *Streptomyces* strains in glycerol casein medium for 10 days on Rotary shaker at 30^{o}C.

Preparation of Newly K Bacterial Strains

Three bacterial K strains namely Histidineless, Argnineless and Phenylalanineless were produced by exposing the wild *Streptomyces*, sp. to ultraviolet rays for 1 to 5 minutes.

Inoculation Process

Each vine inoculated with 5,10 and 20 ml from cell suspension diluted in 2-liters water and then irrigated with vine.

Natural source of feldspar (10.1% K_2O) was purchased from Al-Ahram Company for Egyptian Natural fertilizers, Giza. It was applied in the form of finely (100-mesh) ground natural products.

Field experiment

This study was carried out during the two consecutive seasons of 2015 and 2016 on sixty uniform in vigour 8-years old Superior grapevines grown in a private vineyard where the soil texture is clay (Table 1) and well water drained since water table depth is not less than two meters. The chosen vines are planted at 2 x 3 meters apart. Cane pruning system was followed at the first week of January leaving 84 eyes per vine (on the basis of six fruiting canes x 12 eyes plus six renewal spurs x two eyes) with the assistance of Gable shape supporting system. The vines were irrigated through surface irrigation system using Nile water.

Except those dealing with the present treatments (all sources of K) and biofertilization, all the selected vines (60 vines) received the usual horticultural practices which are commonly used in the vineyard.

This study included the following ten treatments:

1. K as 100% Mineral Potassium (MK) (240 g potassium sulphate/vine (48% K_2O) alone **(Shaaban, 2014)**.
2. K as 75% MK (180 g potassium sulphate/ vine) + 25% feldspar (297.0 g/vine) (10.1% K_2O) + $strain_1$ (Histidineless) at 5 ml/ vine.
3. N as 50% MK (120 g potassium sulphate/ vine) + 50% feldspar (594.0 g/vine) (10.1% K_2O) + $strain_1$ (Histidineless) at 10 ml/ vine.
4. K as 25% MK (60 g potassium sulphate/ vine) + 75% feldspar (891.0 g/vine) (10.1% K_2O) + $strain_1$ (Histidineless) at 20 ml/ vine.
5. K as 75% MK (180 g potassium sulphate/vine) + 25% feldspar (297.0 g/vine) (10.1% K_2O)+ $strain_2$ (Argnineless) at 5 ml/ vine.
6. K as 50% MK (120 g potassium sulphate/ vine) + 50% feldspar (594.0 g/vine) (10.1% K_2O) + $strain_2$ (Argnineless) at 10 ml/ vine.
7. K as 25% MK (60 g potassium sulphate/ vine) + 75% feldspar (891.0 g/vine) (10.1% K_2O)+ $strain_2$ (Argnineless) at 20 ml/ vine.
8. K as 75% MK(180 g potassium sulphate/ vine) + 25% feldspar (297.0 g/vine) (10.1% K_2O) + $strain_3$ (Phenylalanineless) at 5 ml/ vine.
9. K as 50% MK (120 g potassium sulphate/ vine) + 50% feldspar (594.0 g/vine) (10.1% K_2O) + $strain_3$ (Phenylalanineless) at 10 ml/ vine.
10. K as 25% MK (60 g potassium sulphate/ vine) + 75% feldspar (891.0 g/vine) (10.1% K_2O) + $strain_3$ (Phenylalanineless) at 20 ml/ vine.

Each treatment was replicated three times, two vines per each. Potassium sulphate (48% K_2O) as a source of mineral K was divided into three unequal batches, 25% before bloom (3rd week of March), 25% just after berry setting (3rd week of April) and 50% one month later (3rd week of May). The slow release K fertilizer

Table 1. Analysis of the tested soil

Constituent	Value
Particle size distribution	
Sand (%)	7.0
Silt (%)	21.5
Clay (%)	71.5
Texture	Clay
pH(1:2.5 extract)	7.95
EC (1 :2.5 extract) (dsm^{-1}) 1 cm/25^{o}C.	0.81
OM (%)	2.01
$CaCO_3$ (%)	2.41
Total N (%)	0.11
Available P (Olsen, ppm)	3.11
Available K (ammonium acetate, ppm)	405.9

(feldspar (10.1 K_2O) was added once just before growth start (1st week of March) in shallow holes under vine canopy (20 cm apart from vine trunk). All K bacterial strains (Histidineless, Argnineless, Phenylalanineless) were added with feldspar and covered with moist soil. All the selected 60 vines were received botanical compost (Table 2) at fixed rate (0.25 kg/vine). It was added once just after winter pruning (3rd week of January) 50 cm far from the vine trunk in drenches (50 × 50 × 50 cm dimensions). Analyses of botanical compost are given in Table 2.

Randomized complete block design (RCBD) was followed, where the experiment consisted of ten treatments, each treatment was replicated three times, two vines per each.

During both seasons the following parameters were recorded:

1-Growth aspects *i.e.,* main shoot length (cm.), number of leaves/shoot, leaf area $(cm)^2$ (**Ahmed and Morsy, 1999**), pruning wood weight/vine, wood ripening coefficient (**Bouard, 1966**) and cane thickness (cm.).

2-Percentage of total carbohydrates in the canes (**Smith *et al*., 1956**).

3- Leaf chemical components *i.e.* a , b and total chlorophylls and total carotenoids (mg/1.0g FW) (**Von- Wettstein, 1957**) as well as percentages of N, P, K, Mg, Ca and S **(Wilde *et al*., 1985; Balo *et al*., 1988)**.

4- Berry setting percentage.

5-Yield/vine expressed as weight (kg.) and number of clusters per vine as well as cluster weight (g) and dimensions (length and shoulder) (cm).

6- Shot berries percentage.

7-Physical and chemical characteristics of the berries *i.e.* berry weight (g) and dimensions (longitudinal and equatorial) (cm), TSS (%), total acidity, reducing sugars (%) (**Lane and Eynon, 1965; AOAC, 2000**)

8- Total counts of bacteria in the soil was counted (cfug)/1.0g soil **(Cochran, 1950)**.

Statistical ananluysis was done and treatment means were compared using new LSD at 5% according to (**Rao, 2007**).

Table 2. Analyses of plant compost

Parameter	Value
Cubic meter weight (kg.)	600.0
Moisture (%)	29.0
Organic matter (%)	30.7
Organic carbon (%)	28.56
pH (1: 2.5 extract)	6.20
EC (dsm^{-1}) (1: 2.5 extract)	1.20
C/N ratio	14.28
Total N (%)	2.0
Total P (%)	1.02
Total K (%)	1.21
Total Ca (%)	1.25
Total Mg (%)	1.30
Total Fe (ppm)	18.5
Total Mn (ppm)	37.55
Total Zn (ppm)	43.22
Total Cu (ppm)	17.40

RESULTS AND DISCUSSION

Number and Percentage of *Streptomyces* sp. Survivals after Treatment with Different Doses of Ultraviolet (UV) Irradiation

It is clear from the results obtained in Table 3 and Fig. 1 that exposing *Streptomyces* wild type to Ultraviolet (UV) for 1, 2, 3, 4 and 5 minutes was accompanied with decreasing number and percentages of *Streptomyces* sp. survivals compared to control (non treatment).

Exposing *Streptomyces* sp. to five minutes gave zero number and percentages of survival.

The aim of using ultraviolet (UV) irradiation on wild type of *Streptomyces* sp. was induction and isolation of different mutants.

Results of detection and identification of mutants induced by UV treatment are presented in Table 4. Results showed that percentages of detected mutants in wild type of *Streptomyces* sp. In addition, the eighty five stable mutants of wild type were identified as, five Phenylalanineles, six Tyrosineless, eight Tryptophaneless, thirteen Histidineless, fifteen Argnineless, eighteen Methionineless, twelve Threonineless and eight Valineless.

Vegetative Growth Characteristics

It is clear from the obtained results in Table 5 that supplying the vines with K as 50 to 75% mineral K + feldspar at 25 to 50% + any one of the three K bacterial strains (Histidineless, Argnineless, Phenylalanineless) each at 5 to 10 ml/vine, significantly enhanced the six growth characteristics *i.e.* main shoot length, number of leaves/shoot, leaf area, wood ripening coefficient, pruning wood weight and cane thickness relative to employing potassium *via* 100% MK or when K was added as 25% MK even with the application of feldspar and any one of the three K strains.

Table 3. Number and percentage of *Streptomyces* sp. survival after different doses of Ultraviolet irradiation

Organism	Time Exp. (Mint)	Number and percentage of survival	
		Number	(%)
Streptomyces sp.	Control	8800	100.00
	1	5270	59.88
	2	3177	36.10
	3	1005	11.42
	4	499	5.67
	5	0.00	0.00

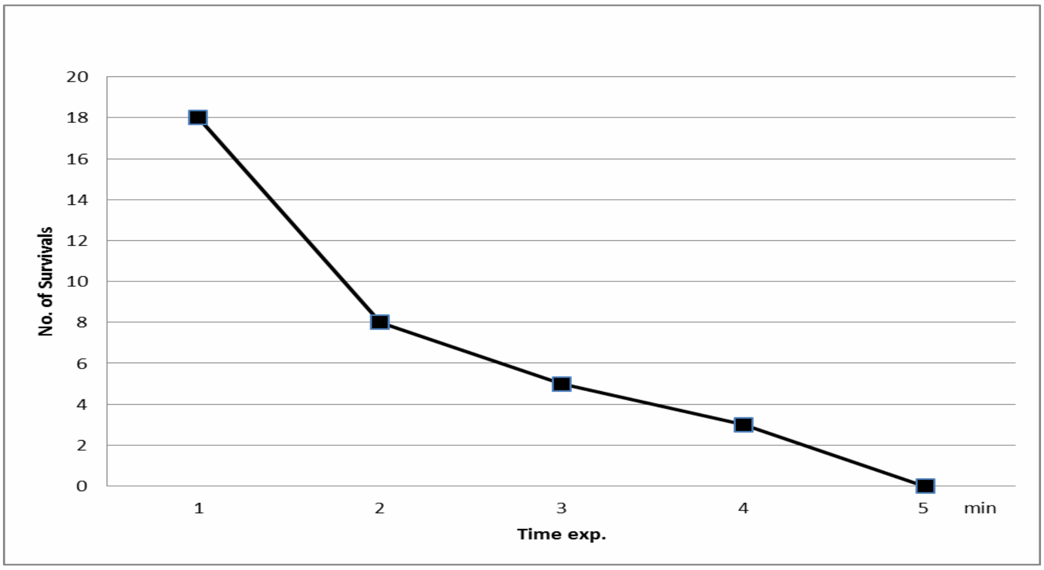

Fig. 1. Number and percentage of *Streptomyces* sp. survival after different doses of ultravioIt irradition

Table 4. Percentage and number of characterization single mutants resulted by dosage of Ultraviolet in *Streptomyces* sp.

Time (Mint)	No. of colonies tested	No. of mutants	Mutants freq. (%)	Number and percentage of characterization							
				Ph.ala$^-$	Tyros.$^-$	Trypt.$^-$	His.$^-$	Arg.$^-$	Meth.$^-$	Thr.$^-$	Valine.$^-$
1	265	3	1.13	1	0	0	1	0	1	0	0
2	317	10	3.15	1	2	1	1	2	1	1	1
3	387	40	10.33	3	4	4	5	7	9	5	3
4	295	32	10.84	0	0	3	6	6	7	6	4
5	0	0	0	0	0	0	0	0	0	0	0

Table 5. Effect of replacing mineral K fertilizers partially by using the slow release K fertilizer (feldspar) enriched with different K microbial strains on some vegetative growth characteristics of Superior grapevines during 2015 and 2016 seasons

Treatment	Shoot length (cm.)		No. of leaves/shoot		Leaf area $(cm)^2$		Wood ripening coefficient		Pruning wood weight (kg.)		Cane thickness (cm.)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
100 % Mineral Potassium (MK)	99.7	101.8	18.0	18.0	97.0	96.9	0.55	0.57	1.99	2.09	0.87	0.90
75% MK + 25% K feldspar + $strain_1$ at 5 ml/vine	116.9	119.0	28.0	27.0	110.0	109.8	0.78	0.80	2.51	2.60	1.25	1.29
50% MK + 50% K feldspar+ $strain_1$ at 10 ml/vine	118.9	121.0	30.0	29.0	112.0	111.9	0.85	0.87	2.62	2.71	1.41	1.44
25% MK + 75% K feldspar + $strain_1$at 20 ml/vine	95.0	96.9	17.0	16.0	94.9	95.0	0.51	0.53	1.96	2.05	0.85	0.88
75% MK + 25% K feldspar + $strain_2$ at 5 ml/vine	101.9	104.0	20.0	19.0	99.9	100.0	0.59	0.61	2.11	2.20	0.91	0.94
50% MK + 50% K feldspar + $strain_2$ at 10 ml/vine	105.5	107.5	22.0	22.0	103.0	102.9	0.64	0.66	2.20	2.29	0.95	0.98
25% MK + 75% K feldspar + $strain_2$ at 20 ml/vine	92.1	94.1	15.0	14.0	91.0	90.8	0.47	0.49	1.84	1.94	0.77	0.80
75% MK + 25 % K feldspar + $strain_3$ at 5 ml/vine	109.9	112.0	24.0	23.0	105.9	106.0	0.67	0.69	2.31	2.40	1.01	1.04
50% MK + 50% K feldspar + $strain_3$ at 10 ml/vine	112.0	113.9	26.0	25.0	108.0	107.9	0.74	0.76	2.41	2.50	1.14	1.17
25% MK + 75% K feldspar + $strain_3$ at 20 ml/vine	93.9	96.0	16.0	16.0	92.9	93.0	0.49	0.51	1.89	1.98	0.80	0.83
New LSD at 5%	0.8	0.6	2.0	2.0	1.1	0.9	0.03	0.02	0.05	0.06	0.03	0.04

$Strain_1$= Histidineless $Strain_2$= Argnineless $Strain_3$= Phenylalanineles

The stimulation on these characteristics was significantly related to reducing percentage of MK from 75 to 50% and at the same time increasing the percentage of feldspar from 25 to 50% and the levels of K strains from 5 to 10 ml/vine. Reducing inorganic from 50 to 25% of the suitable K as significant reduction on these growth aspects even with using feldspar and potassium strains. The best K strain in this respect was arranged in descending order as follows Histidineless, Phenylalanineless and Argnineless. The maximum values of shoot length (118.9 and 121.0 cm), number of leaves/ shoot (30.0 and 29.0), leaf area (112.0 and 111.9 cm^2), wood ripening coefficient (0.85 and 0.87), pruning wood weight (2.62 and 2.71 kg) and cane thickness (1.41 and 1.44 cm) were recorded on the vines that received K as 50% MK + 50% feldspar + 10 ml/vine Histidineless. The lowest value was recorded by the vines subject to K as 25% MK + 75% feldspar + K strain Argnineless at 20 ml/vine. These results were true during the both seasons.

Leaf and Cane Chemical Component

Fertilizing the vines with K as 50 to 75% mineral K + feldspar at 25 to 50% + any K bacterial strain each at 5 to 10 ml/vine, significantly enhanced the eleven chemicals namely cane total carbohydrates (%) as well as percentages of N, P, K, Mg, Ca and S (%), chlorophylls a and b, total chlorophylls and total carotenoids in the leaves relative to employing potassium *via* 100% MK or when K was added as 25% MK even with the application of feldspar and any one of the three K strains (Tables 6 and 7).

The promotion on these chemical componance was significantly in proptional to reducing percentage of MK from 75 to 50% and at the same time increasing the percentage of feldspar from 25 to 50% and the levels of K strains from 5 to 10 ml/vine. The best K strains in this respect was arranged in descending order as follows Histidineless, Phenylalanineless and Argnineless. The maximum values of cane total carbohydrates (16.6 and 16.5%), N (2.11 and 2.10%), P (0.281 and 0.285%), K (1.84 and 1.87%), Mg (0.84 and 0.84%), Ca (2.41 and 2.40%), S (0.99 and 1.00%), chlorophyll a (2.69 and 2.74 mg/1gfw.), chlorophyll b (2.41 and 2.45 mg/1gfw.), total chlorophylls (5.10 and 5.19 mg/1gfw.) and total carotenoids (1.89 and 1.90) were recorded on the vines that received K as 50% MK + 50% feldspar + 10 ml/vine Histidineless. Supplying the vines with K as 25% MK + 75% feldspar + K strain Argnineless at 20 ml/vine gave the lowest value. These results were true during both seasons.

Percentage of Berry Setting (%), Yield and Cluster Aspects

It is worth to mention from the obtained results in Table 8 that amending the vines with K as 50 to 75% mineral K + feldspar at 25 to 50% + any K bacterial strains (Histidineless, Argnineless, Phenylalanineless) each at 5 to 10 ml/vine, significantly enhanced berry setting (%), yield expressed in weight (kg) and number of clusters/vine as well as weight, length and shoulder of cluster relative to supplying potassium *via* 100% MK or when K was added as 25% MK even with the application of feldspar and any one of the three K strains.

The promotion on these parameters was significantly in proptional to reducing percentage of MK from 75 to 50% and at the same time increasing the percentage of feldspar from 25 to 50% and the levels of K strains from 5 to 10 ml/vine. The best K strains in this respect was arranged in descending order as follows Histidineless, Phenylalanineless and Argnineless. The maximum values were recorded on the vines that received K as 50% MK + 50% feldspar + 10 ml/vine Histidineless. Supplying the vines with K as 25% MK + 75% feldspar + K strain Argnineless at 20 ml/ vine gave the lowest value. Number of clusters/vine in the first season was significantly unaffected by the present treatments. These results were true during both seasons.

Percentage of Shot Berries

It is worth to mention from the obtained results in Table 9 that supplying the vines with K as 50 to 75% mineral K (potassium sulphate) + the slow release K fertilizer (feldspar) at 25 to 50% + any one of the three bacterial strains (Histidineless, Argnineless, Phenylalanineless) each at 5 to 10 ml/vine was followed by controlling the percentage of shot berries relative to amending potassium *via* 100% MK or

Table 6. Effect of replacing mineral K fertilizers partially by using the slow release K fertilizer (feldspar) enriched with different K microbial strains on cane total carbohydrates and percentages of leaf contents of N, P, K, Mg, Ca and S of Superior grapevines during 2015 and 2016 seasons

Treatment	Cane total carbohydrates (%)		Leaf N (%)		Leaf P (%)		Leaf K (%)		Leaf Mg (%)		Leaf Ca (%)		Leaf S (%)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
100 % Mineral potassium (MK)	13.6	13.6	1.59	1.60	0.111	0.107	1.37	1.41	0.61	0.61	2.01	2.00	0.69	0.70
75% MK + 25% K feldspar + strain$_1$ at 5 ml/vine	16.0	16.0	1.99	2.00	0.271	0.275	1.79	1.83	0.80	0.81	2.33	2.32	0.96	0.97
50% MK + 50% K feldspar+ strain$_1$ at 10 ml/vine	16.6	16.5	2.11	2.10	0.281	0.285	1.84	1.87	0.84	0.84	2.41	2.40	0.99	1.00
25% MK + 75% K feldspar + strain$_1$at 20 ml/vine	13.7	13.6	1.55	1.56	0.105	0.104	1.91	1.95	0.54	0.55	1.97	1.98	0.65	0.65
75% MK + 25% K feldspar + strain$_2$ at 5 ml/vine	13.9	14.0	1.66	1.65	0.141	0.146	1.44	1.49	0.64	0.65	2.06	2.05	0.75	0.76
50% MK + 50% K feldspar + strain$_2$ at 10 ml/vine	14.2	14.2	1.72	1.71	0.151	0.156	1.51	1.55	0.68	0.69	2.10	2.11	0.80	0.81
25% MK + 75% K feldspar + strain$_2$ at 20 ml/vine	12.9	13.0	1.46	1.46	0.096	0.098	1.57	1.61	0.47	0.48	1.91	1.92	0.59	0.60
75% MK + 25% K feldspar + strain$_3$ at 5 ml/vine	14.9	15.0	1.79	1.80	0.211	0.216	1.64	1.68	0.72	0.73	2.17	2.18	0.86	0.86
50% MK + 50% K feldspar + strain$_3$ at 10 ml/vine	15.4	15.5	1.85	1.85	0.241	0.246	1.68	1.72	0.76	0.76	2.25	2.26	0.92	0.93
25% MK + 75% K feldspar + strain$_3$ at 20 ml/vine	13.3	13.3	1.51	1.51	0.102	0.101	1.72	1.75	0.49	0.50	1.94	1.96	0.62	0.63
New LSD at 5%	0.6	0.5	0.05	0.06	0.08	0.09	0.05	0.06	0.03	0.04	0.04	0.05	0.05	0.06

Strain$_1$= Histidineless   Strain$_2$= Argnineless   Strain$_3$= Phenylalanineless

Table 7. Effect of replacing mineral K fertilizers partially by using the slow release K fertilizer (feldspar) enriched with different K microbial strains on leaf pigments (mg/1g Fw) of Superior grapevines during 2015 and 2016 seasons

Treatment	Chlorophyll a (mg/1g FW)		Chlorophyll b (mg/1g FW)		Total chlorophylls (a+b) (mg/1g FW)		Total carotenoids (mg/1g FW)	
	2015	2016	2015	2016	2015	2016	2015	2016
100% Mineral potassium (MK)	2.01	2.05	1.66	1.70	3.67	3.75	1.59	1.61
75% MK + 25% K feldspar + strain$_1$ at 5 ml/vine	2.55	2.60	2.21	2.25	4.76	4.85	1.85	1.87
50% MK + 50% K feldspar+ strain$_1$ at 10 ml/vine	2.69	2.74	2.41	2.45	5.10	5.19	1.89	1.90
25% MK + 75% K feldspar + strain$_1$at 20 ml/vine	1.99	2.03	1.64	1.69	3.63	3.72	1.55	1.56
75% MK + 25% K feldspar + strain$_2$ at 5 ml/vine	2.11	2.15	1.74	1.79	3.85	3.94	1.64	1.66
50% MK + 50% K feldspar + strain$_2$ at 10 ml/vine	2.20	2.25	1.81	1.85	4.01	4.10	1.68	1.70
25% MK + 75% K feldspar + strain$_2$ at 20 ml/vine	1.91	1.96	1.56	1.60	3.47	3.56	1.47	1.49
75% MK + 25% K feldspar + strain$_3$ at 5 ml/vine	2.31	2.36	1.91	1.95	4.22	4.31	1.74	1.76
50% MK + 50% K feldspar + strain$_3$ at 10 ml/vine	2.41	2.46	2.11	2.15	4.52	4.61	1.79	1.81
25% MK + 75% K feldspar+ strain$_3$ at 20 ml/vine	1.97	2.02	1.62	1.66	3.59	3.68	1.51	1.53
New LSD at 5%	0.07	0.07	0.07	0.08	0.08	0.07	0.04	0.05

Strain$_1$= Histidineless Strain$_2$= Argnineless Strain$_3$= Phenylalanineless

Table 8. **Effect of replacing mineral K fertilizers partially by using the slow release K fertilizer (feldspar) enriched with different K microbial strains on percentage of berry setting, yield as well as weight, length and shoulder of cluster of Superior grapevines during 2015 and 2016 seasons**

Treatment	Berry setting (%)		No. of clusters/vine		Yield/vine (kg)		Cluster weight (g)		Cluster length (cm)		Length cluster shoulder (cm)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
100% Mineral potassium (MK)	9.8	10.0	25.0	26.0	9.42	10.10	377.0	388.0	19.1	18.9	9.1	9.0
75% MK + 25% K feldspar + $strain_1$ at 5 ml/vine	13.0	13.2	25.0	28.0	10.50	12.07	420.0	431.0	21.0	20.9	11.0	10.9
50% MK + 50% K feldspar+ $strain_1$ at 10 ml/vine	13.9	14.1	25.0	29.0	10.75	12.79	430.0	441.0	21.9	22.0	11.4	11.5
25% MK + 75% K feldspar + $strain_1$at 20 ml/vine	9.8	10.0	26.0	27.0	9.93	10.70	382.0	396.0	18.8	18.9	8.8	8.9
75% MK + 25% K feldspar + $strain_2$ at 5 ml/vine	10.4	10.7	25.0	26.0	9.52	10.17	381.0	391.0	19.4	19.5	9.5	9.5
50% MK + 50% K feldspar + $strain_2$ at 10 ml/vine	11.0	11.3	25.0	27.0	9.75	10.80	390.0	400.0	19.6	19.7	9.9	10.0
25% MK + 75% K feldspar + $strain_2$ at 20 ml/vine	9.0	9.2	25.0	26.0	9.40	10.06	376.0	387.0	18.3	18.4	8.4	8.5
75% MK + 25% K feldspar + $strain_3$ at 5 ml/vine	11.6	11.8	25.0	27.0	9.85	10.94	394.0	405.0	20.1	20.1	10.3	10.4
50% MK + 50% K feldspar + $strain_3$ at 10 ml/vine	12.1	12.3	26.0	28.0	10.66	11.79	410.0	421.0	20.5	20.6	10.6	10.7
25% MK + 75% K feldspar + $strain_3$ at 20 ml/vine	9.4	9.6	26.0	27.0	9.85	10.53	379.0	390.0	18.6	18.5	8.6	8.36
New LSD at 5%	**0.6**	**0.5**	**NS**	**2.0**	**0.15**	**0.17**	**6.1**	**6.5**	**0.5**	**0.6**	**0.4**	**0.3**

$Strain_1$= Histidineless $Strain_2$= Argnineless $Strain_3$= Phenylalanineless

Table 9. Effect of replacing mineral K fertilizers partially by using the slow release K fertilizer (feldspar) enriched with different K microbial strains on percentage of shot berries as well as physical characteristics of the berries of Superior grapevines during 2015 and 2016 seasons

Treatment	Shot berries (%)		Berry weight (g)		Berry longitudinal (cm)		Berry equatorial (cm)	
	2015	2016	2015	2016	2015	2016	2015	2016
100% Mineral potassium (MK)	11.0	10.9	3.4	3.3	2.06	2.07	1.88	1.90
75% MK + 25% K feldspar + $strain_1$ at 5 ml/vine	6.0	5.9	3.6	3.7	2.10	2.11	1.92	1.93
50% MK + 50% K feldspar+ $strain_1$ at 10 ml/vine	5.0	4.9	3.9	4.0	2.15	2.16	1.96	1.97
25% MK + 75% K feldspar + $strain_1$at 20 ml/vine	11.9	12.0	4.2	4.3	2.19	2.19	1.99	2.00
75% MK + 25% K feldspar + $strain_2$ at 5 ml/vine	10.0	9.9	5.0	4.9	2.31	2.32	2.11	2.12
50% MK + 50% K feldspar + $strain_2$ at 10 ml/vine	9.0	8.9	5.3	5.4	2.35	2.36	2.15	2.16
25% MK + 75% K feldspar + $strain_2$ at 20 ml/vine	13.0	12.9	5.6	5.6	2.38	2.39	2.19	2.19
75% MK + 25% K feldspar + $strain_3$ at 5 ml/vine	8.0	7.9	4.2	4.3	2.20	2.21	2.00	2.01
50% MK + 50% K feldspar + $strain_3$ at 10 ml/vine	7.0	6.9	4.6	4.7	2.25	2.25	2.05	2.06
25% MK + 75% K feldspar + $strain_3$ at 20 ml/vine	12.4	12.3	4.8	5.0	2.28	2.28	2.09	2.09
New LSD at 5%	1.0	1.1	0.2	0.2	0.4	0.3	0.3	0.4

$Strain_1$= Histidineless $Strain_2$= Argnineless $Strain_3$= Phenylalanineless when K was added as 25% MK even with the application of feldspar and any one of the three K strains.

The reduction on such undesirable phenomenon was significantly in proptional to reducing percentage of MK from 75 to 50% and at the same time increasing the percentage of feldspar from 25 to 50% and the levels of K strains from 5 to 10 ml/vine. The best K strains in this respect was arranged in descending order as follows Histidineless, Phenylalanineless and Argnineless. The minimum values on percentage of shot berries (5.0 and 4.9%) were recorded on the vines that received K as 50% MK + 50% feldspar + 10 ml/vine Histidineless. Supplying the vines with K as 25% MK + 75% feldspar + K strain Argnineless at 20 ml/vine gave the highest value. These results were true during the both seasons.

Some Physical and Chemical Characteristics

It can be stated from the obtained results in Tables 9 and 10 that fertilizing the vines with K as 25 to 75% mineral K (potassium sulphate) + the slow release K fertilizer (feldspar) at 25 to 75% + any one of the three bacterial strains (Histidineless, Argnineless, Phenylalanineless) each at 5 to 20 ml/vine, was very significantly effective in improving quality of the berries in terms of increasing weight, longitudinal and equatorial of berry, TSS (%), reducing sugars (%) and decreasing total acidity (%) compared with supplying potassium *via* 100% MK.

The promotion on berries quality was significantly in proptional to reducing percentage of MK from 100 to 25% and at the same time increasing the percentage of feldspar from 25 to 75% and the levels of K strains from 5 to 20 ml/vine. The best K strains in this respect was arranged, in ascending order as follows Argnineless, Phenylalanineless and Histidineless. The best results with regard to physical of the berries were obtained due to supplying the vines with K as 50% MK + 50% feldspar + 10 ml/vine Histidineless. Supplying the vines with K as 25% MK + 75% feldspar + 20 ml/vine strain Argnineless gave satisfactory promotion on chemical quality of the berries. These results were true during the both seasons.

Total Counts of Bacteria in the Soil

It is obvious from the results in Table 10 that fertilizing the vines with K through 25 to 75% mineral K fertilizer + 25 to 75% slow release K fertilizer (feldspar) + any one of the three K strains (Histidineless, Argnineless, Phenylalanineless) each at 5 to 20 ml/vine, significantly enhanced total counts of bacteria in the soil rather than the application of K as 100% mineral. The increase in the total counts of bacteria was significantly associated with reducing the percentage of mineral K from 100 to 25% as well as increasing the percentages of feldspar from 0.0 to 75% and K bacterial strains from 0.0 to 20 ml/vine.

The stimulation was significantly associated with using K strains *i.e.* Argnineless, Phenylalanineless and Histidineless, in ascending order. The maximum values of total count of bacteria in the soil (144.9 and 145.9 cfug) were recorded when the vines received K as 25% MK+ 75% feldspar + 20 ml Histidineless bacterial strain/vine. The lowest values, (123.0 and 124.3 cfug) were recorded when the vines were supplied with K as 100% mineral K. These results were true during both seasons.

Economical Study for the Recommended Treatment if it Applied in one faddan

As shown in Table 11 net profit gained by the application of the recommended treatment (50% Mineral K+ 50% feldspar + 10 ml strain Histidineless) if it applied in one faddan contains 700 vines reached 12376 (LE) and 17046 (LE) while in the control vines (100% MK) reached 9360 (LE) and 10280 (LE) during both seasons, respectively. The increase on net profit due to application of the recommended treatment over the control reached 3016 (LE) and 6766 (LE) during both seasons, respectively.

Table 10. Effect of replacing mineral K fertilizers partially by using the slow release K fertilizer (feldspar) enriched with different K microbial strains on some chemical berry characteristics and total counts of bacteria of Superior grapevines during 2015 and 2016 seasons

Treatment	TSS (%)		Total acidity (%)		Reducing sugars (%)		Total count of bacteria (cfug)/1 g soil	
	2015	2016	2015	2016	2015	2016	2015	2016
100 % Mineral potassium (MK)	17.1	16.9	0.679	0.671	14.7	14.6	123.0	124.3
75% MK + 25% K feldspar + strain$_1$ at 5 ml/vine	17.5	17.6	0.655	0.666	15.2	15.3	141.0	142.3
50% MK + 50% K feldspar+ strain$_1$ at 10 ml/vine	17.8	17.9	0.640	0.644	15.5	15.6	143.0	144.4
25% MK + 75% K feldspar + strain$_1$at 20 ml/vine	18.1	18.2	0.670	0.624	15.7	16.0	144.9	145.9
75% MK + 25% K feldspar + strain$_2$ at 5 ml/vine	19.1	19.2	0.550	0.555	16.8	16.9	125.0	126.0
50% MK + 50% K feldspar + strain$_2$ at 10 ml/vine	19.5	19.6	0.530	0.533	17.1	17.0	129.9	128.9
25% MK + 75% K feldspar + strain$_2$ at 20 ml/vine	19.8	19.8	0.510	0.513	17.4	17.3	131.0	132.0
75% MK + 25% K feldspar + strain$_3$ at 5 ml/vine	18.3	18.4	0.609	0.614	16.0	15.9	135.9	136.9
50% MK + 50% K feldspar + strain$_3$ at 10 ml/vine	18.7	18.8	0.580	0.585	16.4	16.5	137.0	138.3
25% MK + 75% K feldspar + strain$_3$ at 20 ml/vine	19.0	19.1	0.566	0.565	16.7	16.8	138.9	140.0
New LSD at 5%	0.3	0.3	0.017	0.018	0.2	0.3	1.9	2.1

Strain$_1$= Histidineless Strain$_2$= Argnineless Strain$_3$= Phenylalanineless

Table 11. Economical study for the recommended treatment if it applied on one faddan

Recommended treatment	2015	2016
Costs Hort. Practices (LE)	17000	18000
Costs of potassium sulphate (LE)	650	650
Costs of feldspar (LE)	171	171
Costs of Histidineless bio (LE)	154	154
Total costs (LE)	17975	18975
Yield/fad. (tons)	7.53	8.95
Price of yield/fad. (LE)	30120	35800
Net profit (LE)	12145	16825
Control		
Costs Hort. Practices (LE)	17000	18000
Yield/fad. (tons)	6.59	7.07
Price of yield/fad. (LE)	26360	28280
Net profit (LE)	9360	10280
Increase over control (LE)	2785	6545

* Price of ton grapes in the first (2015) and second season (2016) were 4000 (LE).

DISCUSSION

K-solubilizing bacteria are able to release potassium from insoluble minerals**.** In addition, researchers have discovered that K-solubilizing bacteria can provide beneficial effects on plant growth through suppressing pathogens and improving soil nutrients. For example, certain bacteria can weather silicate minerals to release potassium, silicon and aluminum, and secrete bio-active materials to enhance plant growth. These bacteria are widely used in biological K-fertilizers and biological leaching. The considerable populations of KSB are present in rhizospheric soils which promote the plant growth **(Lian *et al*., 2002; Gundala *et al*., 2013; Zhang *et al*., 2013).**

It is generally accepted that the major mechanism of mineral K-solubilization is the action of organic acids synthesized by rhizospheric microorganism. Productions of organic acids results in acidification of the microbial cell and its surroundings environment which promote the solubilization of mineral K. Silicate bacteria was found to resolve potassium, silicon and aluminum from insoluble minerals **(Aleksandrov *et al.*, 1967)**.

Silicate bacteria exert beneficial effects upon plant growth and yield. The KSB can promote K-solubilization and is very important to enhance the fertility status of soils. Rhizospheric microorganisms contribute directly and indirectly to the physical, chemical and biological parameters of soil through their beneficial or detrimental activities. Rhizospheric bacteria helps in soil processes such as exudation of soluble compounds, storage and release of nutrients, mobilization and mineralization of nutrients, soil organic matter decomposition and solubilization of K. A wide range of bacteria namely *Pseudomonas*, *Burkholderia, Acidothiobacillus ferro, Bacillus mucilaginosus*, *Bacillus edaphicus*, *Bacillus circulans*, *and Paenibacillus* spp have been reported to release potassium in accessible form from K-bearing minerals in soils (**Liu *et al*., 2012)**.

These potassium solubilizing bacteria (KSB) were found to dissolve potassium, silicon and aluminium from insoluble K-bearing minerals such as micas, illite and orthocalses, by excreting organic acids which either directly dissolved rock K or chelated silicon ions to bring K into the solution. Inoculation with the KSB have been reported to exert beneficial effects on growth of pepper and cucumber. Similary, inoculation of maize and wheat plants with *Bacillus mucilaginosus*, *Azotobacter chroococcum*, and *Rhizobium* resulted in significant higher mobilization of potassium from waste mica, which in turn acted as a source of potassium for plant growth. Therefore, potassium solubilizing bacteria are extensively used as biofertilizer in Korea and China as significant areas of cultivated soils in these countries are deficiency in soil-available K. Thus application of K-solubilizing bacteria as biofertilizers for agriculture improvement can reduce the use of agrochemicals and support ecofriendly crop production **(Han *et al.*, 2006; Singh *et al.*, 2010)**.

The beneficial effect of organic manures in enhancing growth, vine nutritional status and fruiting of Superior grapevines might be attributed to their important roles on enhancing soil fertility, organic matter, availability of all nutrints as well as the biothensises of nutral hormones, B vitamins and antibiotics as well as lowering soil pH and salinity (**Mengel *et al.*, 2001**).

Previous studies showed that using K biofertilizers besides mineral, slow or fast release fertilizers had an announced promotion on fruiting of fruit crops **(Sheng and Huang, 2002; Ahmed and Abada, 2012; Shaheen *et al.*, 2013; Allam, 2014; Shaaban, 2014; Ahmed *et al.*, 2016)**.

Conclusion

For promoting yield of Superior grapevines, it is advised to supply the vines with K as 50% inorganic K plus 50% feldspar and 10 ml strain Histidineless . Supplying the vines with K as 25% mineral K + 75% feldspar + 20 ml strain Argnineless gave the best results with regard to berries quality.

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تحسين امتصاص العناصر وإنتاجية عنب السوبيريور باستخدام بعض سلالات البكتريا الميسرة للبوتاسيوم

عمر فتحي داخلى[1] - ماهر خيري يواقيم[2]

١- قسم الوراثة - كلية الزراعة - جامعة المنيا - مصر

٢- قسم بحوث العنب - معهد بحوث البساتين - مركز البحوث الزراعية - الجيزة - مصر

أجريت تجربة معملية خلال موسمى ٢٠١٥، ٢٠١٦ وذلك لتحسين إمتصاص البوتاسيوم وإنتاجية عنب صنف السوبيريور بإنتاج ثلاث سلالات بكتيرية جديدة ميسرة للبوتاسيوم (Histidineless, Argnineless and Phenylalanineless) وذلك بتعريض بكتريا *Streptomyces* لتركيزات مختلفة من الأشعة الفوق بنفسجية لمدة دقيقة إلي خمسة دقائق ولقد تم استخدام هذه السلالات مع السماد البوتاسي بطئ التحلل (الفلسبار) كبديل جزئى للسماد المعدنى البوتاسي، كان هناك تحسن ملحوظ فى جميع صفات النمو الخضرى والصبغات وعناصر النيتروجين والفوسفور والبوتاسيوم والكالسيوم والكبريت والنسبة المئوية لعقد الحبات وكمية المحصول وخصائص الجودة للحبات وذلك عند استخدام التسميد البوتاسي على هيئة ٥٠ إلى ٧٥% سماد بوتاسي معدني، سماد الفليسبار بنسبة مئوية ٢٥ إلي ٥٠% وأى سلالة من سلالات البوتاسيوم بمعدل من ٥ إلى ١٠ مل وذلك بالمقارنة بمعاملة التسميد البوتاسي المعدنى فقط، وكانت أفضل سلالة بكتيرية ميسرة للبوتاسيوم هى (Histidineless)، (Phenylalanineless) واحتلت السلالة (Argnineless) المركز الأخير فى هذا الصدد، ولأجل تحسين كمية المحصول فى العنب السوبيريور فانه ينصح بتسميد الكرمات بالبوتاسيوم المعدني بنسبة ٥٠% مع الفلسبار بنسبة ٥٠% مع استخدام السلالة ذات العوز الغذائي لل (Histidineless) بمعدل ١٠ مل للكرمة، أما تسميد الكرمات بالبوتاسيوم علي أساس ٢٥% بوتاسيوم معدنى مع فلسبار بنسبة ٧٥% مع السلالة ذات العوز الغذائي لل (Argnineless) بمعدل ٢٠ مل للكرمة فانه يعطى أفضل النتائج بخصوص خصائص الجودة للحبات.

المحكمون :

١- أ.د. أحمد عطية العايـــدي أستاذ الفاكهة – كلية الزراعة – جامعة طنطا.

٢- أ.د. صفاء عبدالغني أحمد أستاذ الفاكهة المتفرغ – كلية الزراعة – جامعة الزقازيق.