

Original Research Article

A consortium of soil bacteria mediates the partial replacement of mineral fertilizer for sustainable grapevine cultivation in sandy soil.

4

Abstract

6

The demand for chemical fertilizers in agriculture has increased to deal with the present global population increase. However, the excessive use of chemical fertilizers can be reduced by applying biofertilizers as an eco-friendly tool. Plant growth-promoting rhizobacteria (PGPR) has an essential need in terms of fertilizer savings and promoting plant yield. Here, we study the effect of using three (PGPR) bacterial strains “*Bacillus nakamurai* MSRH1, *Bacillus pacificus* MSRH3, *Paenibacillus polymyxa* MSRH5”, integrated with chemical fertilizers (40, 60, 80, 100% need based NPK) on vegetative growth, yield production, and quality of table grapes ‘Flam Seedless’ grown in sand soil during two successive seasons of 2020 and 2021, with a preliminary trial season in 2019. Our results show that amending grapes with NPK in combination with the consortium of three strains led to significant improvement in colonized vines compared to a single application of 100% NPK. Results showed that bacterial consortium combined with 80% and 60% NPK mineral fertilizer had more positive effects than un-inoculated vines in growth parameters, cluster characteristics, yield/vine and berry quality in the two growing seasons. Besides, N, P and K concentrations of leaf petiole, total leaf chlorophyll content, and carbohydrates in canes were significantly enhanced by bacteria consortium with 80% and 60% PK chemical fertilizers. PGPR significantly increased total bacterial count, N₂-fixing, P- solubilizing and K-solubilizing bacteria in soil treated with the three strains of bacteria plus mineral fertilizer. In addition, dehydrogenase and phosphatase activity in the rhizosphere soil were also increased in treatments inoculated with strains plus mineral fertilizer. The field study results showed that PGPR approach has potential and can be considered as a crop management strategy to increase the yield and quality of grapes, reduce chemical fertilization and subsequent environmental pollution, and could be useful in terms of sustainable production.

Keywords: Grapevines, chemical fertilizer, PGPR, growth, yield.

1. 32 Introduction

Grape (*Vitis vinifera*, L.), family *Vitaceae*, is one of the top ranked crops worldwide because of its nutritional value, multilabel use and profitable income [1]. In Egypt, Flam

Seedless grape has been classified as the second cultivated fruit trees, with 810.3 million m² extension in the last few years [2]. However, the green revolution aims to increase plant production per unit area, which consequently depends on chemical fertilizers to provide crops with major essential nutrients [3]. In this respect, synthetic fertilizers have been reported to enhance soil fertility with nutrients which its deficiencies [4,5]. Furthermore, plants require three essential nutrients, nitrogen, phosphorous and potassium to maintain good growth performance, balance plant physiological function and quality parameters [6]. The most essential crop nutrients in agricultural systems are nitrogen (N), phosphorus (P), and potassium (K) [7].-Nitrogen is the main factor of the plant cell and vital to the metabolic processes, such as photosynthesis, and core element for amino acids in plant structures [8]. Nitrogen is also a factor in the development of DNA, which contains the genetic blueprint of the plant – it's a component of nucleic acid [9]. Like nitrogen, phosphorus plays an essential role in biosynthesis, respiration, energy storage, cell division, translocation of carbohydrates, and a crucial element for both DNA and RNA [10]. Besides, phosphorus improves the overall crop quality and supports plants throughout their life cycle, stimulating root development, increasing the strength of stems and stalk, supporting flowering and the production of seeds as well as contributing to an earlier and more uniform crop maturity [11,12,13]. Potassium, an important element, increases the production of carbohydrates and proteins, catalyzes the activity of some enzymes, stimulates the synthesis and accumulation of thiamin and riboflavin and is critical for the activity of guard cells [14]. In addition, potassium helps to regulate the level of water in the plant, affecting both the uptake of water by the roots and its loss through evaporations; it can therefore improve a plants tolerance to drought [15,16]. On the other hand, excessive application of NPK fertilizers beyond crops' demand not only can cause harmful environmental and ecosystem impacts, but also affect crop quality and human health [17,18]. To achieve maximum benefits in terms of fertilizer savings and better development, plant growth promoting rhizobacteria (PGPR) inoculation technology can be considered as an effective sustainable nutritional crop supplementation for alleviating the use of chemical fertilizers [19,20], improving crop yield and to sustain soil health [21,22]. PGPRs are an important component of the rhizosphere of many plants that affect plant growth either by different direct or indirect mechanisms [23]. It promotes plant growth by creating phytohormones [24] and vitamins [25], improving root branching and root diameter [26], altering systematic resistance against various phytopathogens [27] are some of PGPR impacts in agriculture system. In addition, PGPR, as microbial inoculants,

have a positive impact on soil biology and it can be recognized as a good strategy for recovering semi-arid areas and degraded ecosystems [28]. PGPR and their attributes with plants are well known and starting to be exploited commercially in many crops [29,30]. To overcome excessive NPK application, we investigate the impact of using a well characterized soil bacteria isolated from Egyptian soil as biological biofertilizer on growth of Grapes. A PGPRs consortium (*Bacillus nakamurai* MSR11, *Bacillus pacificus* MSR13, and *Paenibacillus polymyxa* MSR15), on growth, production, and quality of colored Table Grapes 'Flam Seedless' supplied with four levels of NPK fertilizers in sand soil.

2. Materials and methods

2.1. Plant materials and NPK application

The present work was conducted during the two successive seasons of 2020 and 2021, with a preliminary trial season 2019, at a ten-years-old Flam Seedless vineyard (*V. vinifera*, L) (Fig.1), grown in sand soil with drip irrigation system, at El-Khatatba region, Minya Governorate (30° 22' 16.7"N, 30° 46' 40.5" E", Egypt (Fig.2) . The physical and chemical properties of the soil (Table 1) were determined according to [31]. Forty free disease and uniform size vines, cultivated at 2 x 3 m apart and trellised by the Spanish Pantone shape system, were selected. Vines were pruned, in the last week of December of 2020 and 2021 seasons, respectively, with a load of 68 buds per vine. All vines received the mineral fertilizers were added as recommended by ministry agriculture as follow: Thirty units (P) of calcium superphosphate and 50 kg of Sulphur fertilizers (S) were added once at the beginning of vegetative growth stage. One hundred units of potassium sulfate (K) and 60 units of ammonium nitrate (N) were divided to three doses (25% at the beginning of vegetative growth, 50% after fruit set, and 25% after harvest stages). Fifty kilograms of magnesium sulfate (Mg) were added at 10 kg/ month. Fifty kilograms of calcium nitrate (Ca) were divided to two doses (before bloom and after fruit set stage). Mineral NPK fertilizers were applied as single treatment (100% NPK, control) or as 40, 60, and 80% of NPK in combination with bacterial consortium.

97

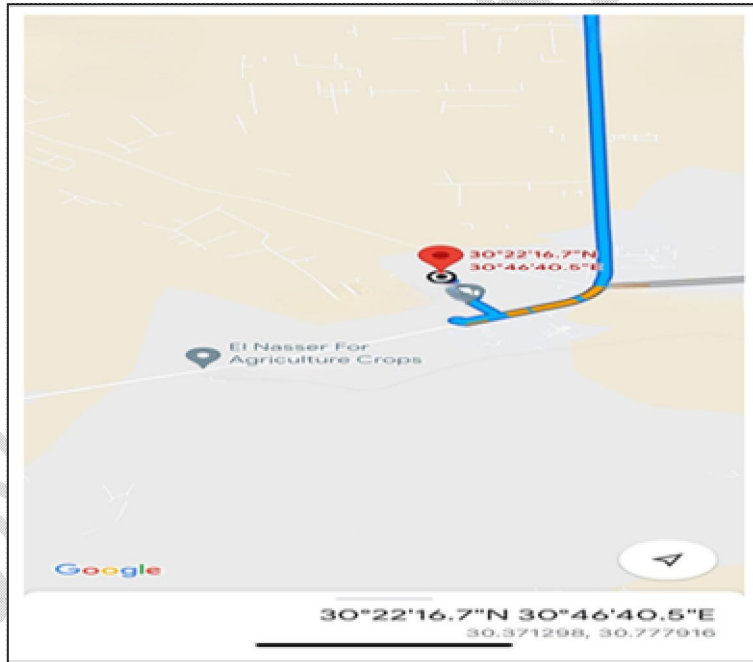
98

99

100



102
103
104
105
Fig.1.103 photograph (A) of Flam Seedless vineyard (*V. vinifera*, L) grown with Spanish Parron shape system and (B) before winter pruning



106
107
108
109
110
111
112
113
114
115

Fig.2. Elnasser farm's direction for agriculture crops

Table 1. Physical and chemical properties of the soil.

Depth of Sample	Soil	PH	EC dS/m	Ca ⁺⁺ (mg/L)	Mg ⁺⁺ (mg/L)	Cl ⁻ (mg/L)	CO ³⁻ (mg/L)	HCO ³⁻ (mg/L)	So ⁴ (mg/L)	K(mg/L)	Na(mg/L)	SAR*
Superficial		8.32	5.15	37	12	15.2	0	5	76	1.7	45.5	9.2
30 cm	Sand	8.32	5.85	42	10	21.9	0	7	44	3.9	16.9	3.3
60 cm		8.32	4.88	40	6	20	0	5	38	2	14.8	3.1
Available nutrients (p.p.m)				P	K	Fe	Zn	Mg	Cu	Caco3 %	O. M. (%)	
	Superficial			24	127	3.3	2.3	2.3	35	3.7	1.2	
	30 cm			22	121	1.89	1.32	1.2	22	2.3	0.87	
	60 cm			18	117	2.6	1.2	1.64	0.1	2.1	0.54	

(*) SAR= sodium adsorption ratio.

2.2. Bacterial growth and inoculum preparation

Bacillus nakamurai MSR11, *Bacillus pacificus* MSR13 and *Paenibacillus polymyxa* MSR15 were provided by Professor Abo-Koura's Microbiology Department, Soil, Water and Environmental Institute (SWERI), Agriculture Research Center (ARC), Giza, Egypt. Briefly, the pre-inoculum for each strain was prepared in agar plates then incubated at 30±2°C for 48h. Later, numerous identical developing colonies were conveyed into a liquid broth medium for 12h at 30±2°C in a rotary shaker at 120 rpm. For inoculum harvesting, each medium was centrifuged (7,000 g for 10 min) then washed twice in potassium phosphate buffer (pH 7.0, 60 mmol). The optical density was measured by spectrophotometer (OD 10⁸ cells/ mL at λ600 nm) [32]. To prepare the inoculums, MSR11, MSR13 and MSR15 were grown individually in nutrient broth medium for 48 h at 28°C, in a rotary shaker incubator at 150 rpm to exponential phase (6x10⁷, 5x10⁶ and 5x10⁶ cfu. ml⁻¹, respectively). Ten ml suspension of each strain was mixed to colonize vine plants at the second week of January.

130

2.3. Characterization of PGPR traits

The three bacterial strains were grown on nutrient broth medium then incubated at 28±2°C for 24 h. Exopolysaccharides (EPS) was determined by [33] while Indole acetic acid (IAA) was determined as described by [34]. Biofilm creation was examined according to [35].

136

2.4. Growth parameters, leaves N, P, K and Chlorophyll content

At the harvest time, shoot length and number of leaves per plant were measured manually. Leaf area meter (Model CI 203, USA) was used to determine leaf area of the 6th and 7th apical leaves. During the flowering stage, ten samples were taken from leaves opposite to cluster to determine N, P and K content in leaf petioles [36] and total chlorophyll content [37].

143

2.5. Bud burst and fertility percentage

Numbers of buds were counted one month after bud burst and the percentage of buds burst were calculated [38] as follows:

Bud burst % = (Number of bursted buds per vine / Total buds per vine) x 100.

While number of clusters per vine were counted and divided by the total number of buds and then the fertility was calculated as follows:

Bud fertility % = (Number of clusters per vine / Total buds per vine) x 100

151

25. Yield

Grape bunches were taken from all possible locations on grapevines so that the samples represent correct yield and quality attributes. In this respect, average cluster weight (g) was measured, while the average yield per vine was calculated as follow:

Yield per vine = number of clusters per vine × average cluster weight

157

25. Berries quality

A sample of 50 berries were selected from each replica and the juice was extracted to measure total soluble solids (TSS, Brix⁰) at room temperature by hand refractometer, while titratable acidity (TA, gram tartaric acid/100 ml juice) was determined by titration with NaOH [39]. Total anthocyanin for the berries skin (mg/100g fw) was calculated according to [40]. Total carbohydrates in fruiting canes were determined calorimetrically [41].

165

26. Wood ripening and pruning weight

At dormant seasons, twelve shoots for each replicated were select to assess the coefficient of wood ripening, which calculated by dividing length of the ripened part by the total length of the shoot [42]. In addition, one year old pruning wood weight per vine (g) was calculated [43].

171

27. Total bacterial count, N-Fixer bacteria, Phosphate and Potassium solubilizing bacteria

Total bacterial count in rhizosphere soil was done as described by [44], then Nitrogen fixing bacteria were counted on glucose mineral media (NFGMM) [45]. P-solubilizing and K-releasing bacteria were calculated on agar medium for 3-5 days at 28°C [46].

177

28. Enzyme activity

Dehydrogenase activity (DHA) ($\mu\text{gTPF g}^{-1}$ dry soil day⁻¹) in rhizosphere soil for each treatment was determined as described by [47]. Alkaline and acidic phosphatases (mg/dry soil) were determined according to [48].

29. Costs and net profit /feddan

Yield per feddan ton (average two seasons) = Yield (kg fruit/vine) x Number of vines/700.

Total costs / feddan (Egyptian pounds) (L.E.) = Treatments (amino acids) costs/ feddan (L.E.) + Costs of cultural practices/ feddan (L.E.).

Total production/ feddan (L.E.) = Yield/ feddan ton X price of one ton.

Net profit / feddan (L.E.) = Total production/feddan (L.E.) - Total costs / feddan (L.E.).

2.12. Statistical Analyses

The experiment was conducted in three replicates, as a completely randomized block design (CRBD). All data were analyzed by ANOVA, least significant difference (LSD) test was used to compare means using the statistical analysis software; CoStat (CoHort Software, USA) version 6.4. The values of probability $p \leq 0.05$ were considered statistically significant based on the least significant difference test.

194

3. Results

3.1. Growth, bud behavior and yield traits

Data in Table (2) reveal significant increases in number of leaves, leaf area, bud burst, bud fertility, yield per vine and cluster weight with 80 and 60% NPK + PGPRs consortium than 100% NPK. Yield and cluster weight recorded 16 and 17% increase, with 80% NPK + PGPRs consortium when compared to control in the two seasons, respectively. Obviously, 40% NPK + PGPRs consortium recorded lower yield component than other treatments. Regarding to growth traits, shoot length revealed no significant difference between treatments. Meanwhile, significant increase in number of leaves was obtained with vines supplied with 80% NPK + PGPRs consortium than other treatments. Similar trend was observed in terms of yield per vines and cluster weight.

206

3.2. Nitrogen, phosphorus, potassium, and leaf chlorophyll

According to data presented in Table (3), content of N, P, K in leaf petiole and total leaf chlorophyll were positively affected by inoculation vine with PGPRs consortium combined with different mineral NPK, than single NPK application. In addition, the three nutrients concentrations were higher in vine treated with 80% NPK + PGPRs consortium followed by vine treated with 60% NPK + PGPRs consortium than control in the two successive growing seasons. In terms of leaf chlorophyll content, better values were observed in leaves of vines treated with PGPRs consortium than control. However, no significant differences between 40, 60, 80% NPK + PGPRs consortium were obtained in two seasons.

Table 2. Effect of the combination between PGPRs and 100, 80, 60, 40 % NPK levels on growth bud behavior and yield of Flam Seedless grapevines.

Treatments	Number of leaves		Shoot length (cm)		Leaf area (cm) ²		Bud burst %		Bud fertility %		Yield (kg)		Cluster weight (g)	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
100% NPK (Control)	30	31	248	250	171	166	89	89	74	75	15.5	16.5	483	517
80% NPK+ PGPR	35	36	253	252	179	175	94	94	79	80	18.1	19.3	567	603
60% NPK+ PGPR	32	35	251	248	170	168	92	93	77	78	16.7	17.9	523	540
40% NPK+ PGPR	30	32	252	251	172	169	79	75	64	68	12.0	11.3	300	327
L.S.D. (0.05)	1.0	1.3	NS	NS	2.3	1.7	1.4	1.0	1.6	1.7	0.92	1.1	28.7	26.7

Table 3 Effect of the combination between PGPRs and 100, 80, 60, 40 % NPK levels on N, P, K contents in leaf petiole and total chlorophyll of Flam Seedless grapevines

Treatments	N (%)		P (%)		K (%)		Chlorophyll (mg/g FW)	
	2020	2021	2020	2021	2020	2021	2020	2021
100% NPK (Control)	2.11	2.25	0.34	0.37	1.43	1.52	36.50	34.40
80% NPK+ PGPR	2.43	2.44	0.54	0.47	1.62	1.64	37.63	35.30
60% NPK+ PGPR	2.40	2.38	0.42	0.45	1.50	1.58	37.13	35.07
40% NPK+ PGPR	2.16	2.37	0.38	0.43	1.44	1.53	37.10	34.97
L.S.D. (0.05)	0.01	0.02	0.01	0.01	0.01	0.08	0.32	1.28

219
220
221
222
223

3.3. Quality of berries

No significant differences observed between berries in terms of TSS under PGPRs inoculation than control (Table 4). On the other hand, vines treated with 80% NPK + PGPR consortium followed by vine treated with 60% NPK + PGPRs consortium revealed significant increases in values of titratable acidity and anthocyanin concentration, respectively.

230

3.4. Growth promoting activities of the consortium

The three strains were tested for their plant growth promoting characteristics including Exopolysaccharides (EPS), IAA and biofilm formations (Table 5). MSRH1 showed highest production for EPS and biofilm followed by MSRH3 and MSRH5, respectively, while higher IAA was produced with MSRH5 than other strains.

236

Table 37 Effect of the combination between PGPRs and 100, 80, 60, 40 % NPK levels on quality of Flam Seedless berries.

Treatments	T.S.S (Brix ^o)		Titratable acidity (%)		Anthocyanin (mg /100g FW)	
	2020	2021	2020	2021	2020	2021
100% NPK (Control)	16.67	17.00	0.80	0.60	34.27	33.50
80% NPK+ PGPR	17.00	17.67	0.60	0.60	38.37	37.47
60% NPK+ PGPR	16.67	17.33	0.70	0.60	36.57	35.57
40% NPK+ PGPR	16.67	17.33	0.60	0.60	35.83	34.37
L.S.D. (0.05)	NS	NS	0.07	0.05	1.8	2.5

239

Table 40 Exopolysaccharides (EPS), IAA and biofilm formations by three bacterial strains.

241

Strain	Exopolysaccharide (EPS)	IAA production	Biofilm formation
<i>Bacillus nakamurai</i>	+++	+	+++
<i>Bacillus pacificus</i> MSRH3	++	+	++
<i>Paenibacillus polymyxa</i> MSRH5	+	++	+

242

243

3.5. Population of soil bacteria upon consortium inoculation

Three strains were used as potential plant growth promoting bacteria, and consortium to produce a complex inoculant and effect of the mix were monitored. Forty-five days post-burial of Flam Seedless grapevines, the population of N₂-fixing, P-solubilizing, and K-solubilizing bacterial growth in soil were powerfully enhanced with 80% NPK + bacteria in the two seasons (Table 6). Moreover, the N₂-fixing, P- and K-solubilizing bacteria were higher in vine treated with consortium in combination with NPK fertilizer than control.

252

253

UNDER PEER REVIEW

Table 6. Effect of PGPRs consortium inoculation on population of soil bacteria of Flam Seedless grapevines

Treatments	Total count of bacteria		N ₂ -fixing bacteria	P-solubilizing bacteria	K-solubilizing bacteria	N ₂ -fixing bacteria	P-solubilizing bacteria	K-solubilizing bacteria
	(x 10 ⁶ cfu g ⁻¹ soil)							
	2020	2021	2020			2021		
100% NPK (Control)	66.2 ± 0.61	73.8 ± 0.53	25.9±0.67	28.8 ± 1.7	15.4± 0.53	33.1± 0.66	29.77± 9.77	17.5± 0.52
80% NPK+ PGPR	90.3 ± 1.91	94.9± 4.1	43.4 ± 1.2	56.5± 0.6	52.6±2.8	49.2 ± 1.0	58.53± 0.85	53.3± 0.59
60% NPK+ PGPR	80.0 ± 1.86	82.5 ± 1.30	33.6 ± 1.05	42.5 ± 5.5	26.4±3.09	39.6± 1.0	43.80±43.80	28.53± 0.61
40% NPK+ PGPR	76.8± 0.53	78.4± 0.50	33.1± 0.7	29.27± 1.15	33.1± 0.7	35.1±0.40	32.6±0.90	34.7± 0.50

254

3.5. Enzymatic activities in soil

The Dehydrogenase activity (DHA), acidic phosphatase and alkaline phosphatase activity in the soil treated with bacterial consortium was significantly increased with 80% NPK+ PGPRs consortium followed by 60%, respectively, in comparison to control (Table 7). No significant differences were found between controls and 40% NPK inoculated with bacterial consortium in the two growing seasons (Table 7).

261

Table 7 Effect of PGPRs consortium inoculation on enzymatic activities in soil of Flam Seedless grapevines

Treatments	Dehydrogenase activity ($\mu\text{g TPF g}^{-1}$ dry soil $^{-1}$ day $^{-1}$)		Acidic Phosphatase activity ($\mu\text{g pnp g}^{-1}$ soil h $^{-1}$)		Alkaline phosphatase activity ($\mu\text{g pnp g}^{-1}$ soil h $^{-1}$)	
	2020	2021	2020	2021	2020	2021
100% NPK (Control)	50.6	68.3	75.7	84.9	52.3	55.2
80% NPK+ PGPR	76.8	91.1	90.9	91.6	65.8	68.5
60% NPK+ PGPR	72.7	87.8	86.9	89.2	59.4	62.5
40% NPK+ PGPR	51.7	70.3	81.5	71.1	64.0	64.5
L.S.D. (0.05)	4.4	2.1	1.5	1.2	1.6	1.3

264

3.7. Wood ripening, pruning weight and carbohydrates in canes

Application of bacterial consortium plus NPK mineral fertilizer increased significantly ripening wood, pruning wood weight and carbohydrates in canes in two growing seasons (Table 8). It is obvious that application 80% NPK mineral fertilizer + PGPRs showed the highest value for pruning wood weight, coefficient of wood ripening and carbohydrates in canes compared to other treatments.

271

Table 8 Effect of PGPRs consortium inoculation on pruning wood weight, coefficient of wood ripening and carbohydrates in canes of Flam Seedless grapevines.

274

Treatments	Weight of pruning/vine (g)		Wood ripening coefficient		Carbohydrates in canes (%)	
	2020	2021	2020	2021	2020	2021
100% NPK (Control)	3067	3250	0.74	0.75	23.00	24.20

80% NPK+ PGPR	3367	3467	0.84	0.86	28.10	29.20
60% NPK+ PGPR	3333	3433	0.84	0.85	25.70	28.10
40% NPK+ PGPR	3300	3400	0.81	0.84	25.80	27.00
L.S.D. (0.05)	121	577	0.02	0.01	1.05	0.71

275

3.8. Costs and net profit /feddan

It is clear from the obtained data in (Table 9) that Application three strains (PGPRS) for partial replacement of Mineral NPK fertilizer of Flame seedless grapevines with mineral NPK fertilizer gave the best net profit/ feddan as compared with (100 % mineral NPK control). In addition, Application of 80% mineral NPK fertilizer mixed with NPK bacteria gave the highest values of net profit / feddan which recorded 10603 (L.E.) over control as average two seasons.

283

Table 9 Costs and net profit /feddan of three strains (PGPR_s) for partial replacement of Mineral NPK fertilizer applications of Flame seedless as average two seasons 2020 and 2021 seasons

286

Treatments		Costs of *cultural practice without mineral NPK / fed. (L.E.)	Costs of mineral NPK/ fed. (L.E.)	Costs of treatments NPK bacteria costs/fed. (L.E.)	Total costs / fed. (L.E.)	/Yield /fed Ton	Total production /fed. (L.E.)	Net profit / fed. (L.E.)	Net profit / fed. over control (L.E.)
T1	100 % mineral NPK	25000	10000	0	35000	11.200	67200	32200	0
T2	80% mineral NPK+ NPK bacteria	25000	8000	2800	35800	13.100	78603	42803	10603
T3	60% mineral NPK+ NPK bacteria	25000	6000	2800	33800	12.103	72618	38818	6618
T4	40% mineral NPK+ NPK bacteria	25000	4000	2800	31800	11.648	69888	38088	5888

Cultural practices such as (Fertilizers, Pesticides, fungicides, Irrigation and Labour)

- Pesticide /1 liter from NPK bacteria = 20 (L .E.)
- NPK bacteria 70 x 2doses = 140 liter = 2800 (L.E.) / feddan
- 0.2 feddan = 700 vines
- Pesticide one ton from yield = 6000 (L .E.)

49 Discussion

294 Flam Seedless is a popular grape grown in Egypt for local consumption and
 295 export markets. Meanwhile, excessive, and continuous application of chemical fertilizers
 296 increases the production costs, decrease fruit quality, and harm soil chemical and
 297 biological properties causing an environmental pollution [49]. In this respect,
 298 bio-fertilizers including the PGPR have reported to improve nutritional rank, yield, physical
 299 and chemical properties of colonized soil [50,51, 52].

300 Plant growth promoting rhizobacteria (PGPR) are bacterial strains that reside in
 301 the plant rhizosphere, interact with plants roots, and affect their growth and productivity
 302 by diverse mechanisms [53]. In this study, three strains “*Bacillus nakamurai* MSRH1,
 303 *Bacillus pacificus* MSRH3, *Paenibacillus polymyxa* MSRH5” were selected based on
 304 their effectiveness in colonizing plant root and production of Exopolysaccharides (EPS), IAA
 305 and biofilm, as shown in Table (5). Clearly, the combination between 80% NPK and
 306 PGPR consortium caused an improvement in vegetative growth characters and bud
 307 behavior of inoculated vines in comparison to control, 60 and 40% NPK levels,
 308 respectively, in the two growing seasons. Application of PGPR consortium significantly
 309 increased number of leaves, branch length and leaf area, which agrees with [54] on Flam
 310 Seedless. These positive effects of PGPR inoculation on growth traits of colonized vines
 311 are the result of stimulating plant systemic resistance, root branching, production of IAA,
 312 and enhancing soil fertility [55,65, 57]. Several reports indicated the capability of PGPR
 313 in complete or partial replacement of chemical fertilizers to increase growth and yield of
 314 profitable crops [58]. For instance, supplying grapevines with NPK fertilizers at 50% in
 315 combination with PGPR improved growth and fruiting over the application of mineral N
 316 alone [59]. In addition, application of PGPR consortium plus 80, 60 and 40% of NPK
 317 fertilizer significantly increased maturity parameters and cluster characteristics of
 318 colonized Flam Seedless than un-colonized vines Table (2). The increased fertility in the
 319 grapevine may be caused by apical dominance and vegetative vigor found in Flam
 320 Seedless [60]. In connection, [61] reported that complex bacteria significantly increased
 321 yield and clusters weight of Flam Seedless grapevines as compared with mineral
 322 fertilization.

323 Our results are in line with [62] on Pomegranate, [63] on mango, [64] on citrus,
 324 [65] on olive, [66] on strawberry. The authors reported that bio-fertilizer containing N-
 325 fixing bacteria combined with mineral NPK were more active in enhancing accumulation

of N, P and K in leaves of colonized plant than un-colonized. In addition, the beneficial effect of the bacterial strains might be related to its effect on increasing nitrogen fixation, creation of growth promoting substances or organic acids and improving nutrient uptake [67]. In conclusion, the previous beneficial effect of PGPR on growth and vine nutritional status surely reflected on improving berry setting and cluster weight consequently the yield [68].

These positive effects of PGPR inoculation on growth and nutritional status of Flam Seedless grapes led to an increase in pruning wood weight, coefficient of wood ripening and carbohydrates in canes, as shown in table (8). This may be explained based on fact that soil microorganisms excrete a range of hormones, growth substances and antibiotics that promote plant growth [69]. Overall studies of the present study proposed that among the most studied PGPR, have several reported properties, as well as nitrogen fixation [70] phosphorus solubilization [71] antibiotic synthesis [72] and phytohormone production [73].

According to the effect of PGPR application on quality of grape berries, our results show the highest values of total soluble solids, total acidity and anthocyanin were found in berries of plants colonized with PGPR combined with mineral NPK, mainly at 80% NPK, if compared with single NPK application Table (4). These results are in harmony with [74] who found that bio-fertilizer significantly increased TSS and decreased juice acidity of Flam Seedless grapevines. In addition, inoculation with *G. versiforme* improved the percentage of titratable acidity content compared to non-inoculated [75].

This increase may be explained because of the healthy superior of treated vine, as shown in Table (3), which increases nutrient content and chlorophyll activity of colonized plants [76]. In this respect, it is important to mention that bacterial strains in the present study had especially positive effect on the stimulus of grape leaf chlorophyll content as compared with un-inoculated plants Table 3. Likewise, [77] revealed that the maximum chlorophyll index was obtained in sugarcane leaves by application of *Pseudomonas sp.* The increments in chlorophyll could be related to the enhancement in element uptakes induced by bacteria [78].

Furthermore, supplying superior grapevines with *Azospirillum brasilense* proved to be very effective in stimulating carbohydrates percentage [68], while application of bio-fertilizers for five-year-old seedless grapevine increased carbohydrate content than control [54]. It is also worth mentioning that interaction between PGPR and the plant

depend on plants genotype, soil types, and harvest targets [79]. As shown in Table (6), the total bacterial count, N₂-fixing, P-solubilizing, and K-solubilizing bacteria in soil of grapes were powerfully enhanced due to the applied complex inoculant NPK bacteria. These results are harmony with [80] who found that the populations of N₂-fixing bacteria, as well as P-and K-solubilizing bacteria were significantly higher than inoculated kiwi fruit plants. In connection, mineral fertilizer combined with PGPR application recorded high bacteria counts as compared to the control treatment [81,82]. These PGPR in turn lead to the speed of more exudates and plant products for usage by the plant root plus increase rhizosphere bacterial biomass [83]. This trend is supported by obtained increase in enzymatic activities as illustrated in Table (7).

However, evaluation of the enzymatic activity can provide early evidence of the changes in the soil environment, long before the changes of the chemical composition and physical properties of soils. One of the most important groups of soil enzymes is dehydrogenases (DHA) that present in all the live cells of microorganisms [84], and hence are often considered to be the indicators of the general microbial activity of the rhizosphere [85]. DHA plays an essential role for organic matter decomposition and nutrient remobilization of rhizosphere soil [86,87], while soil acidic phosphatase enzyme show an essential role in the mineralization of organic P [88]. The present study showed that Acid or Alkaline phosphatase, and dehydrogenase enzymes in grapes tree rhizosphere were significantly increased by complex application of PGPR consortium with mineral fertilizer Table (7). This might because of the mechanisms of bacteria in civilizing the physical and chemical soil properties, particularly the soil structure, which improve the microbial activity in the soil [89]. In this study, a high number of bacteria were creating to have positive effect on dehydrogenase activity in soil, as high dehydrogenase activity was observed compared to un-inoculated treatments [90]. Previous studies also stated that the N-fixing and P-solubilizing bacterial strains had the capability to be responsible for nutrients and encourage each other by their physical and biochemical activities thus enhancing the physical properties of plants [91]. Therefore, this study suggests that the application of bio fertilizers plus mineral NPK fertilizer could encourage soil microbes to synthesize dehydrogenase and phosphatase and promoting microbial metabolic activity.

390

5. Conclusion and Recommendations

PGPR might contribute to improve plants growth by modulating the physiological and biochemical activities of plants, especially those underlying the acquisition of mineral

nutr394t. In addition, the three strains, namely *Paenibacillus polymyxa* MSR5, *Bacillus*
395*nankaiensis* MSR1 and *Bacillus pacificus* MSR3, had biological adaptation of the
396soil, and there were increasing in the population of bacteria. In this respect, tested
397PGPR consortium could be recommended as a partial substitute of mineral NPK fertilizer
398in vinyard of Flam Seedless grapevine. However, application of 80% NPK mineral
399fertilizer mixed with PGPR was the best practice might help to shrink the use of
400agrochemicals, as also foreseen by the green deal concept for an increasingly sustainable
401agriculture.

References

- 403Imran M, Rauf A, Imran A, Nadeem M, Ahmad Z, Atif M. Health Benefits of
404Grapes Polyphenols. *J. Environ. Agric. Sci.* 2017; 10: 40-51.
- 405Egyptian Ministry of Agriculture. *Agri. Econom.* 2021; 2.
- 406Chandini RK, Kumar R, Om, P. The Impact of Chemical Fertilizers on our
407Environment and Ecosystem. *In: Res. Trends Envir. Sci.* 2019; 71-86.
- 408Souza RD, Ambrosini A, Passaglia LM. Plant growth-promoting bacteria as
409inoculants in agricultural soils. *Genet. Mol. Biol.* 2015; 38: 401-19.
- 410Tsafaras I, Campen JB, de Zwart HF, Voogt W, Al Harbi A, Al Assaf K,
411Abdelaziz ME, Qaryouti M, Stanghellini C. Quantifying the trade-off between
412water and electricity for tomato production in arid environments. *Agric. Water*
413*Manag.* 2022; 271: 107819.
- 414Sharma A, Chetani R. A review on the effect of organic and chemical fertilizers
415on plants. *Int. J. Res. Appl. Sci. Eng. Technol.* 2017; 5: 677- 686.
- 416Chude VO, Malgwi WB, Amapu IV, Ano OA. Manual on Soil Fertility
417Assessment (Ed). Federal Fertilizer Department (FFD) in collaboration with
418FAO/National Special Programme for Food Security, Abuja, Nigeria. 2004.
- 419Martínez-Dalmau J, Berbel J, Ordóñez-Fernández R. Nitrogen Fertilization. A
420Review of the Risks Associated with the Inefficiency of Its Use and Policy
421Responses. *Sustainability* 2021; 13:5625.
- 422Van den Ackerveken GF, Dunn JM, Cozijnsen AJ, RM, Vossen JPM, Van den
423Broek HW J, Wit PJG, M De. Nitrogen limitation induces expression of the
424avirulence gene *avr9* in the tomato pathogen *Cladosporium fulvum*. *Molec. Gen.*
425*Genet.* 1994; 243: 277-285.
- 426Raven, J.A. RNA function and phosphorus use by photosynthetic organisms.
427*Front. Plant Sci.* 2013; 4:536.
- 428Farooq M, Wahid A, Kobayashi N, Fujita D, Basra S M A. Plant drought stress:
429effects, mechanisms and management. *Agron. Sustain. Dev.* 2009; 29: 185-212.
- 430Sharma S, Kumar V, Tripathi RB. Isolation of phosphate solubilizing
431microorganism (PSMs) from soil. *J. Microbiol. Biotechnol. Res.* 2011; 1: 90-95.
- 432Khan M, A Latif M, Arsalan M, Aziz M, Ulla R, Bilal M, Ashgar W, Latif R,
433Ehsan M, Mehmood MT. Enhancing phosphorus bioavailability in maize through
434phosphorus solubilizing fungi. *Intl. J. Agric. Biol.* 2022; 2: 2843-288
- 435Xinxiang Xu, Xin Du, Wang Fen, Jianchuan Sha, Chen Qian, Tian Ge, Zhanling
436Zhu, Shunfeng Ge, Yuanmao Jiang. Effects of Potassium Levels on Plant Growth,
437Accumulation and Distribution of Carbon, and Nitrate Metabolism in Apple
438Dwarf Rootstock Seedlings. *Front. Plant Sci.* 2020; 11: 904.

439 Cakmak I. The role of potassium in alleviating detrimental effects of abiotic
440 stresses in plants. *J. Plant Nutr. Soil Sci.* 2005; 168: 521-530.

441 Hu Wei, Nan Jiang, Jiashuo Yang, Yali Meng, Youhua Wang, Binglin Chen,
442 Wenqing Zhao, Derrick MO, Zhiguo Zhou. Potassium (K) supply affects K
443 accumulation and photosynthetic physiology in two cotton (*Gossypium hirsutum*
444 L.) cultivars with different K sensitivities. *Field Crops Res.* 2016; 196: 51-63.

445 Adesemoye AO, Torbert HA, Klopper JW. Increased plant uptake of nitrogen
446 from 15N-depleted fertilizer using plant growth-promoting rhizobacteria. *Appl.*
447 *Soil Ecolo.* 2010; 46: 54-58.

448 Sainju UM, Ghimire R, Pradhan GP. Nitrogen fertilization I: Impact on crop, soil,
449 and environment. *Nitrogen Fix.* 2019; 9: 1-9.

450 Shahrajabian MH, Chaski C, Polyzos N, Petropoulos SA. Biostimulants
451 Application: A Low Input Cropping Management Tool for Sustainable Farming of
452 Vegetables. *Biomolecules* 2021; 11: 698.

453 Ye H, Wen Y, Chen Z, Zhang T, Li S, Guan M, Zhang Y, Su S. Relationship of
454 Soil Microbiota to Seed Kernel Metabolism in *Camellia oleifera* Under Mulched.
455 2022, *Front. Plant Sci.* 13: 920604.

456 Sood G, Rajesh K, Girish P, Meenakshi D. Effect of Indigenous Plant Growth-
457 Promoting Rhizobacteria on Wheat (*Triticum Aestivum* L.) Productivity and Soil
458 Nutrients. *Comm. Soil Sci. Plant Analysis.* 2019; 2:141-152.

459 Saad MM, Eida AA, Hirt H. Tailoring plant-associated microbial inoculants in
460 agriculture: a roadmap for successful application. *J. Exp. Bot.* 2020; 13:3878-
461 3901.

462 Saber Z, Esmaeili MA, Abbasian A, Heidarzadeh A. Response of Wheat Growth
463 Parameters to Co-Inoculation of Plant Growth Promoting Rhizobacteria (PGPR)
464 and Different Levels of Inorganic Nitrogen and Phosphorus. *World Appl. Sci. J.*
465 2012; 16: 213-219.

466 Synek L, Rawat A, L'Haridon F, Weisskopf L, Saad MM, Hirt H. Multiple
467 strategies of plant colonization by beneficial endophytic *Enterobacter* sp. SA187.
468 *Environ Microbio.* 2021; 10: 6223-6240.

469 Barnawal D, Bharti N, Maji D, Chanotiya CS. ACC deaminase containing
470 *Arthrobacter protophormiae* induces NaCl stress tolerance through reduced ACC
471 oxidase activity and ethylene production resulting in improved nodulation and
472 mycorrhization in *Pisum sativum*. *J. Plant Physio.* 2014; 171: 884-894.

473 Russo RO, Berlyn GP. The Use of Organic Biostimulants to Help Low Input
474 Sustainable Agriculture. *J. Sustain. Agric.* 1991; 1: 19-42.

475 Daler S, Çetin EM. Determination of the effects of plant growth-promoting
476 rhizobacteria applications on Kober 5 BB American grapevine rootstock in lead
477 stress. *J. Biol. Agri. Health.* 2017; 12: 50-55.

478 Yakhin OI, Lubyantsev AA, Yakhin IA, Brown PH. Biostimulants in Plant Science:
479 A Global Perspective. *Front. Plant Sci.* 2017; 7: 2049.

480 Gray EJ, Smith DL. Intracellular and extracellular PGPR: commonalities and
481 distinctions in the plant–bacterium signaling processes. *Soil Biol. Biochem.* 2005;
482 37: 395-412.

483 Syed Nabi RB, Shahzad R, Tayade R, Shahid M, Hussain A, Ali MW, Yun BW.
484 Evaluation potential of PGPR to protect tomato against *Fusarium* wilt and
485 promote plant growth. *PeerJ.* 2021; 16: 9.

486 Richards LA. Diagnosis and Improvement of Saline and Alkali Soils. United
487 States Department of Agriculture, Washington, D.C. USA. 1954.

- 388 Bashan Y. Significance of timing and level of inoculation with rhizosphere
489 bacteria on wheat plants. *Soil Biol. Biochem.* 1986; 18: 297-301.
- 390 Gilickmann E, Dessaux YA. Critical examination of the specificity of the
491 Salkowski reagent for indolic compounds produced by phytopathogenic bacteria.
492 *Appl. Environ. Microbiol.* 1995; 61: 793-796.
- 393 Damery JT, Alexander M. Physiological differences between effective and
494 ineffective strains of rhizobium. *Soil. Sci.* 1969; 103: 209-215.
495
- 396 Christensen GD, Simpson WA, Younger JJ, Baddour L, Barrett FF, Melton DM,
497 Beachey EH. Adherence of coagulase negative Staphylococci to plastic tissue
498 culture plates: a quantitative model for the adherence of Staphylococci to medical
499 devices. *J. Clin. Microbiol.* 1985; 22: 996-1006.
- 500 Cottenie A, Verloo M, Kiekens L, Relgho G, Camerlynck W. Chemical analysis
501 of plant and soil. Lab. Analytical Agrochemist. State Univ. Gent, Belgium. 1982.
- 502 Mackinny, G. Absorption of light by chlorophyll solution. *J. Bio. Chem.* 1941;
503 140: 315- 322.
- 504 Bessis R. Sur Different Models Expression Quantitative De la fertilité chez la
505 vigne Aca. 1960: 828-882.
- 506 Tourky MN, El-Shahat SS, Rizk MH. Effect of Dormex on fruit set, quality and
507 storage life of Thompson seedless grapes (*Banati grapes*). *J. Agric. Sci. Mansoura*
508 *Univ.* 1995; 12: 5139-5151.
- 509 Husia CL, Luh BS. Chichester, C. D. Anthocyanin in free stone peach. *J. Food*
510 *Sci.* 1965; 30: 5-12.
- 511 Du Bois M, Gilles KA, Hamilton JK, Rebers PT, Smith F. Colorimetric Method
512 for Determination of Sugars and Related Substances. *Analytical Chemist.* 1965;
513 28: 350-356.
- 514 Rizk MH, Rizk NA. Effect of Dormex on bud behavior, yield and rate of wood
515 maturity of Thompson seedless grapevine. Egypt. *J. Appl. Sci.* 1994; 9: 525-542.
- 516 Selim HH, Fayek MA, Sweidan AM. Reproduction of Bircher Apple Cultivar by
517 Layering. *Ann. Agri. Sci. Moshtohor.* 1978; 9: 156-166
- 518 Difco Manual. Dehydrated Culture Media and Reagents for Microbiological and
519 Clinical Laboratory Procedure. 10th Eds, Michigan. USA. 1984.
- 520 Zaw KL, San Y, Ei PK, Tin ML, Ma TN, Wai WM. Isolation, Evaluation and
521 Characterization of Free-Living Nitrogen Fixing Bacteria from Agricultural Soils
522 in Myanmar for Biofertilizer Formulation. *Int. J. Plant Biol. Res.* 2018; 6: 1092.
- 523 Zhang J, Liu J, Y, Meng LY, Ma ZY, Tang XY, Cao YY, Leni S. Isolation and
524 characterization of plant growth-promoting rhizobacteria from wheat roots by
525 wheat germ agglutinin labeled with fluoresce in isothiocyanate. *J. Microbio.* 2012;
526 50: 191-198.
- 527 Skujins, J. Enzymes in soil, In: McLaren AD, Peterson GH. (Eds.), *Soil Biochem.*
528 Marcel Dekker, Inc. New York, USA. 1976; 371-414.
- 529 Tabatabai MA. Soil enzymes, In: Page AL, Miller RH, Keeney DR. (Eds.),
530 *Methods of Soil Analysis. Ameri. Soc. Agron.* 1982:903-947.
- 531 Kobua CK, Jou YT, Wang YM. Advantages of Amending Chemical Fertilizer
532 with Plant-Growth-Promoting Rhizobacteria under Alternate Wetting Drying Rice
533 Cultivation. *Agriculture.* 2021; 11: 605.
- 534 El-Naggar AMA. Effect of Organic Farming on Drip Irrigation Grapevine and
535 Soil Chemical Properties, *In Proceed. 2nd Intl Conf. Agri.* 2004: 117-128.

536 Zhang L, Chengxi Yan, Qing Guo, Junbiao Zhang, Jorge Ruiz-Menjivar. The
537 impact of agricultural chemical inputs on environment: global evidence from
538 informetrics analysis and visualization. *Intl. J. Low-Carbon Techn.* 2018; 13: 338-
539 352.

540 Abdelaziz ME, Atia MAM, Abdelsattar M, Abdelaziz SM, Ibrahim TAA,
541 Abdeldaym EA. Unravelling the Role of Piriformospora indica in Combating
542 Water Deficiency by Modulating Physiological Performance and Chlorophyll
543 Metabolism-Related Genes in Cucumis sativus. *Horticulturae.* 2021; 7:399.

534 Sedri MH, Niedbała G, Roohi E, Niazi M, Szulc P, Rahmani HA, Fiezi V.
545 Comparative Analysis of Plant Growth-Promoting Rhizobacteria (PGPR) and
546 Chemical Fertilizers on Quantitative and Qualitative Characteristics of Rainfed
547 Wheat. *Agronomy*, 2022; 12: 1524.

548 Ahmed MF, Mohamed MN. Improved Productivity of Superior Seedless
549 Grapevines Using Irradiated Compost and Bio-Fertilization in the Desert Land
550 Arab. *J. Nucl. Sci. Appl.* 2018; 51: 94-103.

551 Biswas JC, Ladha JK, Dazzo FB. Rhizobia inoculation improves nutrient up take
552 and growth of low land rice. *Soil Sci. Soc. Amr. J.* 2000; 64:1644-1650.

553 Lucy M, Reed E, Glick BR. Applications of free-living plant growth-promoting
554 rhizobacteria. Review Antonie Van Leeuwenhoek. 2004; 86:1-25

555 Crecchio C, Mimmo T, Bulgarelli D, Pertot I, Pii Y, Perazzolli M, Scagliola M,
556 Cesco S. Beneficial soil microbiome for sustainable agriculture
557 production. *Sustain. Agric. Rev.* 2018; 31: 443-481.

558 Scagliola M, Valentinuzzi F, Mimmo T, Cesco S, Crecchio C, Pii Y.
559 Bioinoculants as Promising Complement of Chemical Fertilizers for a More
560 Sustainable Agricultural Practice. *Front. Sustain. Food Sys.* 2021; 4.

561 Shaaban ASA. Effect of organic fertilization on growth and quality of Superior
562 grapevines. *Ph.D. thesis Fac. Agric. Cairo Univ., Egypt.* 2014.

563 Cássia R, Erasmo J, Marco A, Ilda M, Maria C, Alberto K, Otilia B Fertilidade de
564 gemas de videiras „Niagara Rosada“ de acordo com o sistema de condução. *Rev.*
565 *Bras. Fruti. Jaboticabal.* 2006; 28: 136-138.

566 Khalil HA. The Potential of Biofertilizers to Improve Vegetative Growth,
567 Nutritional Status, Yield and Fruit Quality of Flame Seedless Grapevines,
568 American-Eurasian. *J. Agric. Environ. Sci.* 2012; 12: 1122-1127.

569 Aseri GK, Jain N, Panwar J, Rao AV, Meghwal PR. Biofertilizers improve plant
570 growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of
571 Pomegranate (*Punica granatum* L.) in Indian Thar Desert. *Scient. Hortic.* 2008;
572 117:130-135.

573 Ghazi NA. The effect of Arbuscular Mycorrhiza fungi on the establishment of
574 Sour orange (*C. aurantium*) under different levels of phosphorus. *Acta Hort.* 2013;
575 984: 103-110.

576 Haggag LF, Merwad MM, Awad NM, Shahin MFM, Khalil KH, Mahdy HA.
577 Studies on the effect of bacterial mixture and NPK mineral fertilizer on vegetative
578 and chemical properties of "Toffahi" olive seedlings under greenhouse condition.
579 *J. Agric. Technol.* 2015; 11: 1535-15468.

580 Kundu S, Datta P, Mishra J, Rashmi K, Ghosh B. Influence of biofertilizer and
581 inorganic fertilizer in pruned mango orchard cv. Amrapali. *J. Crop Weed.* 2011; 7:
582 100-103.

583 Rueda D, Valencia G, Soria N, Rueda BB, Manjunatha B, Kundapur RR,
584 Selvanayagam M. Effect of *Azospirillum spp.* and *Azotobacter spp.* on the growth

585 and yield of strawberry (*Fragaria vesca*) in hydroponic system under different
586 nitrogen levels. *J. Appl. Pharm. Sci.* 2016; 6: 48-54.
587 Samah YAE. Effect of Biofertilizer on Yield and Berry Qualities of Grapevines.
588 *Ph.D thesis Fac. Agric. Mansoura Univ. Egypt.* 2002.

589

590 Ahmed FF, Dakhly OF, Abada MAM, Uwakiem M Kh. Effect of some
591 azospirillum strains as a partial replacement of inorganic N fertilizers, on yield
592 and quality of Superior grapevines. *Menoufia J. Plant Prod.* 2017; 2: 495-514.
593 Abobatta WFR. Influence of magnetic iron and K-humate on productivity of
594 Valencia orange trees (*Citrus sinensis* L.) under salinity conditions. *Int. J. Sci.*
595 *Res. Agric. Sci.* 2015; 2:108-119.
596 Cakmakci R, Donmez F, Aydın A, Sahin, F. Growth promotion of plants by plant
597 growth-promoting rhizobacteria under greenhouse and two different field soil
598 conditions. *Soil Biol. Biochem.* 2006; 38:1482-148.
599 De Freitas JR, Banerjee MR, Germida JJ. Phosphate solubilizing rhizobacteria
600 enhance the growth and yield but not phosphorus uptake of canola (*Brassica*
601 *napus* L.). *Biol. Fertil. Soils.* 1997; 24:358-364.
602 Rosado AS, Seldin L. Production of potentially novel anti-microbial substance by
603 *Bacillus polymyxa*. *World J Microbiol. Biotech.* 1993;9: 521-52.
604 Timmusk S, Nicander B, Granhall U, Tillberg E. Cytokinin production by
605 *Paenobacillus polymyxa*. *Soil Biol. Biochem.* 1999; 31:1847-185.
606 Khalil HA. The Potential of Biofertilizers to Improve Vegetative Growth,
607 Nutritional Status, Yield and Fruit Quality of Flame Seedless Grapevines,
608 *American-Eurasian J. Agric. Environ. Sci.* 2012; 12: 1122-1127.
609 Salah E, Kamal A, Mourad S, Khadija H, Hassan G. Effects of inoculation with
610 mycorrhizae and the benefits of bacteria on physicochemical and microbiological
611 properties of soil, growth, productivity and quality of table grapes grown under
612 Mediterranean climate conditions. *J. Plant Protect. Res.* 2021; 1427-4345.
613 Fawzi MIF, Shahin MFM, Kandil EA. Effect of bud load on bud behavior, yield,
614 bunch characteristics and some biochemical contents of the cane of crimson
615 seedless grapevines. *J. Amer. Sci.* 2010; 6: 187-194.
616 Pirhadi M, Enayatizamir N, Motamedi H, Sorkheh K. Impact of soil salinity on
617 diversity and community of sugarcane endophytic plant growth promoting
618 bacteria (*Saccharum officinarum* L. var. CP48). *Appl. Ecology Environ. Res.*
619 2017; 16: 725-739.
620 Ali S, Matilla Y, Zeki K, Fikretin S. Growth and mineral acquisition response
621 of grapevine rootstocks (*Vitis* spp.) to inoculation with different strains of plant
622 growth-promoting rhizobacteria (PGPR). *J. Sci. Food Agric.* 2012; 92: 2148-2153.
623 Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N. Biofertilizers function as key
624 player in sustainable agriculture by improving soil fertility, plant tolerance and
625 crop productivity. *Microbiol. Cell Factories.* 2014; 13:66.
626 Hong Shen, Xinhua He, Yiqing Liu, Yi Chen, Jianming Tang, Tao Guo. A
627 Complex Inoculant of N₂-Fixing, P- and K-Solubilizing Bacteria from a Purple
628 Soil Improves the Growth of Kiwifruit (*Actinidia chinensis*) Plantlets. *Front.*
629 *Microbiol.* 2016; 7: 84.
630 Zhao J, Ni T, Li Y, Xiong W, Ran W, Shen B, Shen Q, Zhang R. Responses of
631 Bacterial Communities in Arable Soils in a Rice-Wheat Cropping System to
632 Different Fertilizer Regimes and Sampling Times. *PLoS ONE.* 2014; 9.

833 Das BB, Dkhar MS. Rhizosphere Microbial Populations and Physico Chemical
634 Properties as Affected by Organic and Inorganic Farming Practices. *Am. Eurasian*
635 *J. Agric. Environ. Sci.* 2011; 10:140-150.

636

837 Curyło K, Telesiński A. Use of Phosphatase and Dehydrogenase Activities in
638 the Assessment of Calcium Peroxide and Citric Acid Effects in Soil Contaminated
639 with Petrol. *Open Life Sci.* 2020; 28:15,12-20.

840 Moeskops B, Sukristiyonubowo Buchan D, Sleutel S, Herawaty L, Husen E,
641 Saraswati R, Setyorini D, De Neve S. Soil microbial communities and activities
642 under intensive organic and conventional vegetable farming in West Java,
643 Indonesia. *Appl. Soil Ecol.* 2010; 45: 112-120.

644

855 Kaczyńska G, Borowik A, Wyszowska J. Soil Dehydrogenases as an
646 Indicator of Contamination of the Environment with Petroleum Products. *Water*
647 *Air Soil Pollut.* 2015; 226: 372.

868 Lioussanne L, Perreault F, Jolicoeur M, St-Arnaud M. The bacterial
649 community of tomato rhizosphere is modified by inoculation with arbuscular
650 mycorrhizal fungi but unaffected by soil enrichment with mycorrhizal root
651 exudates or inoculation with *Phytophthora nicotianae*. *Soil Biol. Biochem.* 2010;
652 42: 473-483.

873 Liang Q, Chen H, Gong Y, Yang H, Fan M, Kuzyakov Y. Effects of 15 years of
654 manure and mineral fertilizers on enzyme activities in particle-size fractions in a
655 North China Plain soil. *Euro. J. Soil Biol.* 2014; 60: 112-119.

886 Wang Y, Shi J, Wang H, Lin Q, Chen X, Chen Y. The influence of soil heavy
657 metals pollution on soil microbial biomass, enzyme activity, and community
658 composition near a copper smelter. *Ecotoxicol. Environ. Safe.* 2007; 67: 75-81.

899 Maqshoof A, Zahir AZ, Jamil M, Latif FNM, Akhtar MF. Integrated use of
660 plant growth promoting rhizobacteria, biogas slurry and chemical nitrogen for
661 sustainable production of maize under salt-affected conditions. *Pak. J. Bot.* 2014;
662 46:375-382.

903 Qaiser Jamal, Yong Seong Lee, Hyeon Deok Jeon, and Kil Young Kim. Effect
664 of Plant Growth-Promoting Bacteria *Bacillus amyloliquefaciens* Y1 on Soil
665 properties, Pepper Seedling Growth, Rhizosphere Bacterial Flora and Soil
666 Enzymes. *Plant Protect. Sci.* 2018; 54:129-137.

907 Jha CK, Saraf M. Evaluation of multispecies plant-growth promoting consortia
668 for the growth promotion of *Jatropha curcas* L. *J. Plant Growth Regul.* 2012; 31:
669 588-598.

670