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A Consortium of Soil Bacteria Mediates the Partial Replacement of Mineral Fertilizer for Sustainable Grapevine Cultivation in Sandy Soil

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MMS and TSAEW did study conceptualization. Author HAAK did formal analysis. Author TSAEW did funding acquisition. Author MMS did study investigation. Authors HAAK, TSAEW and MMS performed methodology. Authors HAAK and MEA helped in project administration and supervision. Authors MMS and MEA wrote the original draft of the manuscript. Authors MEA and MMS wrote, reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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ABSTRACT

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 rizobacteria (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, N_2-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. 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 m^2 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 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 semiarid 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* MSRH1, *Bacillus pacificus* MSRH3, and *Paenibacillus polymyxa* MSRH5), on growth, production, and quality of colonized 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, Minufyia 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 Parron 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.

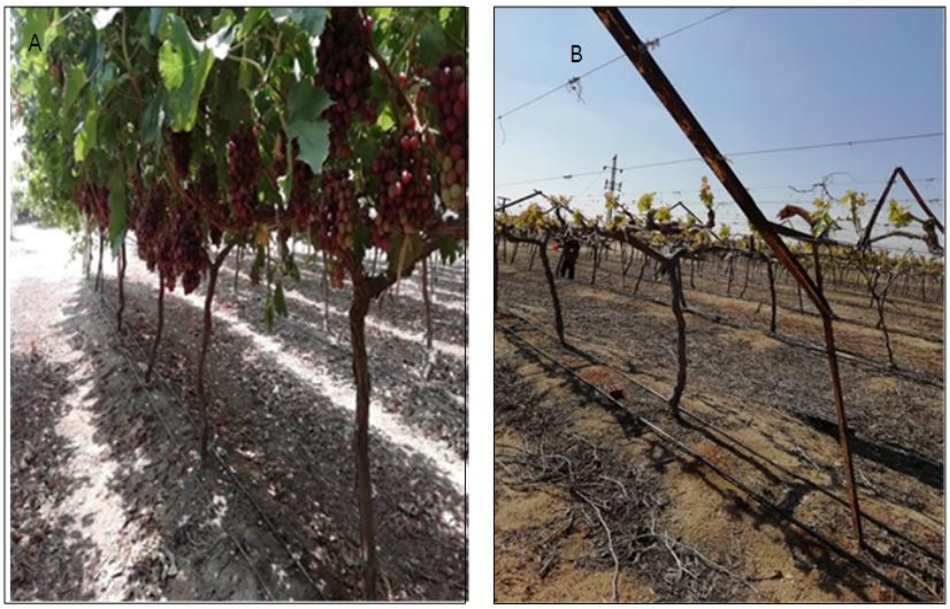

Fig. 1. Photograph (A) of Flam Seedless vineyard (*V. vinifera*, L) grown with Spanish Parron shape system and (B) before winter pruning

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	Sand	8.32	5.15	37	12	15.2	0	5	76	1.7	45.5	9.2
30 cm		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

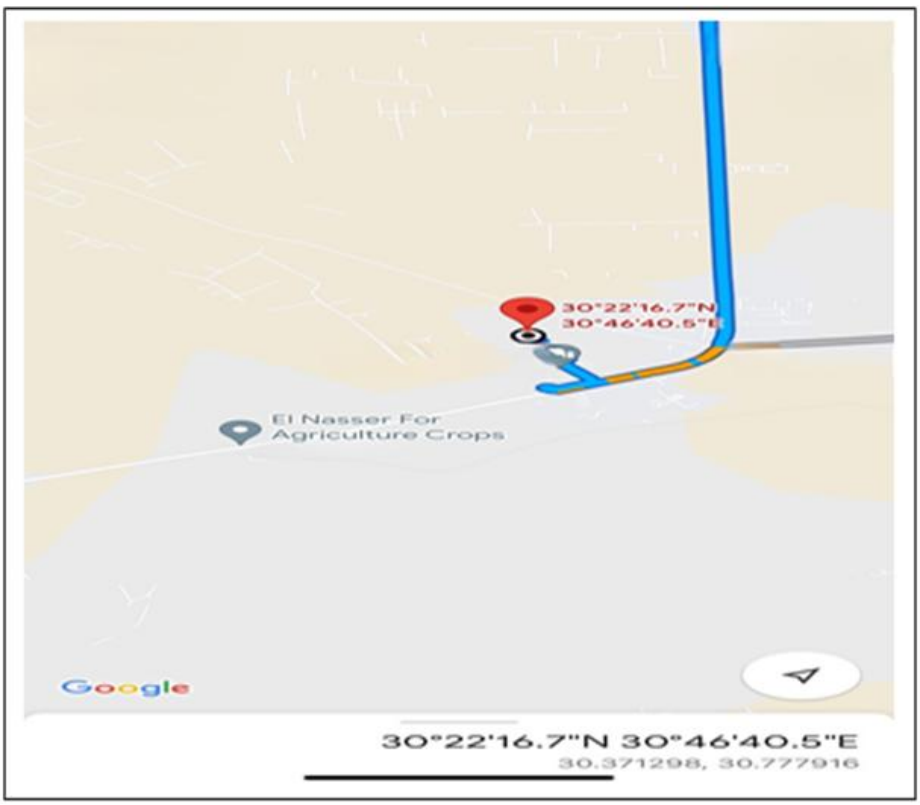

Fig. 2. Elnasser farm's direction for agriculture crops

2.2 Bacterial Growth and Inoculum Preparation

Bacillus nakamurai MSRH1, Bacillus pacificus MSRH3 and Paenibacillus polymyxa MSRH5 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, MSRH1, MSRH3 and MSRH5 were grown individually in nutrient broth medium for 48 h at 28℃, 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 roots at the second week of January.

2.3 Characterization of PGPR Traits

The three bacterial strains were grown on nutrient broth medium then incubated at 28±20C 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].

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 6^{th} and 7^{th} 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].

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

2.6 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

2.7 Berries Quality

A sample of 50 berries were selected from each replica and the juice was extracted to measure total soluble solids (TSS, $Brix^0$) at room temperature by hand refractometer, while titratable acidity (TA, gram tartaric acid/100 ml juice) was determined by titration 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].

2.8 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].

2.9 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^oC [46].

2.10 Enzyme Activity

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

2.11 Costs and Net Profit / Feddan

Yield/ 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 ≤0.05 were considered statistically significant based on the least significant difference test.

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.

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.

3.3 Quality of Berries

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

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.

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 bud burst of Flam Seedless grapevines, the population of N_2-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 N_2-fixing, P- and K-solubilizing bacteria were higher in vine treated with consortium in combination with NPK fertilizer than control.

3.6 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).

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.

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.

4. DISCUSSION

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

Table 2. Effect of the combination between $PGPR_S$ 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

Table 4. Effect of the combination between PGPRs and 100, 80, 60, 40 % NPK levels on quality of Flam Seedless berries

Treatments	T.S.S ($Brix^0$)		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

Table 5. Exopolysaccharides (EPS), IAA and biofilm formations by three bacterial strains.

Strain	Exopolysaccharide (EPS)	IAA production	Biofilm formation
Bacillus nakamurai MSRH1	+++	+	+++
Bacillus pacificus MSRH3	++	+	++
Paenibacillus polymyxa MSRH5	+	++	+

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)		(x10^4 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

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

Treatments	Dehydrogenase activity (µg TPF g dry $soil^{-1}$ day^{-1})		Acidic Phosphatase activity (µg pnp g^{-1} soil h^{-1})		Alkaline phosphatese activity (µg 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

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

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

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

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

Plant growth promoting rhizobacteria (PGPR) are bacterial strains that reside in the plant rhizosphere, interact with plants roots, and affect their growth and productivity by diverse mechanisms [53]. In this study, three strains "*Bacillus nakamurai* MSRH1, *Bacillus pacificus* MSRH3, *Paenibacillus polymyxa* MSRH5" were selected based on their effective in colonizing plant root and production of Exopolysaccharides (EPS), IAA and biofilm, as shown in Table (5). Clearly, the combination between 80% NPK and PGPRs consortium caused an improvement in vegetative growth characters and bud behavior of inoculated vines in comparison to control, 60 and 40% NPK levels, respectively, in the two growing seasons. Application of PGPR consortium significantly increased number of leaves, branch length and leaf area, which agrees with [54] on Flam Seedless. These positive effects of PGPR inoculation on growth traits of colonized vines are the result of stimulating plant systemic resistance, root branching, production of IAA, and enhancing soil fertility [55,56,57]. Several reports indicated the capability of PGPR in complete or partial replacement of chemical fertilizers to increase growth and yield of profitable crops [58]. For instance, supplying grapevines with NPK fertilizers at 50% in combination with PGPR improved growth and fruiting over the application of mineral N alone [59]. In addition, application of PGPR consortium plus 80, 60 and 40% of NPK fertilizer significantly increased maturity parameters and cluster characteristics of colonized Flam Seedless than un-colonized vines Table (2). The increased fertility in the grapevine may be caused by apical dominance and vegetative vigor found in Flam Seedless [60]. In connection, [61] reported that complex bacteria significantly increased yield and clusters weight of Flam Seedless grapevines as compared with mineral fertilization.

Our results are in line with [62] on Pomegranate, [63] on mango, [64] on citrus, [65] on olive, [66] on strawberry. The authors reported that bio-fertilizer containing N-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 by explain 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 plant [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*. These 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, N_2-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 N_2-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 higher bacteria counts as compared to the control treatment [81,82]. These PGPR in turn leads 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 shows 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 a 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.

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 nutrient. In addition, the three strains, namely *Paenibacillus polymyxa* MSRH5, *Bacillus nakamurai* MSRH1 and *Bacillus pacificus* MSRH3, had biological adaptation of the tested soil, and there were increasing in the population of bacteria. In this respect, tested PGPR consortium could be recommended as a partial substitute of mineral NPK fertilizer in vineyard of Flam Seedless grapevine. However, application of 80% NPK mineral fertilizer mixed with PGPR was the best practice might help to shrink the use of agrochemicals, as also foreseen by the green deal concept for an increasingly sustainable agriculture.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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