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Research Article

Impact of Biofertilizer EM1 Combined with NPK on Growth, Flowering, Corm Production, Chemical Composition and Reduction of Mineral Fertilization of Gladiolus Hybrida cv. Rose Supreme

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Abstract | Gladiolus (*Gladiolus grandiflorus* L.) is regarded as one of Egypt's most important flowering bulbs. Gladiolus flower output may be harmed by unbalanced fertilizing over reliance on chemical fertilizers to increase crop productivity, on the other hand, has resulted in health hazards and microbial population issues in soil, as well as being rather expensive and raising the cost of production; these considerations, taken together, stimulate the use of bio-fertilizers. This study's objective was to assess how a biofertilizer EM1 (effective microorganisms) combined with varied rates of the recommended NPK mineral fertilization would affect vegetative growth, flower yield, quality, chemical constituents, and reduce mineral fertilization of NPK on Gladiolus hybrida cv. "Rose Supreme". A pot experiment was undertaken during the planting seasons of 2020 and 2021 in the Antoniades Botanical Gardens, Horticulture Research Institute, Alexandria, Egypt. There are 7 treatments which consist of 3 percent of the suggested soil amendment NPK (50%NPK, 75%NPK, 100%NPK) were applied as a combined with two concentrations of EM1 (Effective Microorganisms) (1% EM1 and 2% EM1). On the other hand, the control treatment used the entire prescribed NPK soil amendment proportion. Biofertilizer (EM1) applications combined with NPK had a substantial impact on enhancement of the characteristics of vegetative growth which investigated, flower and corm characteristics, Photosynthetic pigments in leaves, and nutrient content compared to the control. Plants fertilized with 2% EM1 in combination with either 75 percent or 100 percent NPK exhibited similar benefits, according to the findings in obtaining the highest percentage of increase, especially in the following traits: leaf area, Days to first floret opening, Fresh weight of florets/spik, new corm diameter, new corm fresh weight, chlorophyll and the content of N, P, K, Fe, Mn, and Zn in leaves. It was concluded that using 2% EM1 in combination with either 75 percent or 100 percent NPK significantly improved yield and growth, as well as improved quality, because of increasing bio-N, P, and K production and saving one-fourth of the amount of chemical fertilizer, lowering production costs, and obtaining a high-quality product with decreasing pollution.

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Introduction

Gladiolus (*Gladiolus grandiflorus* L.) is regarded as one of the most prominent flowering bulbs in Egypt. Gladiolus is an important bulbous ornamental plant, perennial geophyte, semi-rustic herbaceous annual bulbous plant belongs to the Iridaceae family. It is employed for potted plants, gardens, and cut flowers in order to meet the growing demand for gladiolus flowers in both the domestic and international markets (Demir and Çelikel, 2019). Plants of gladiolus grown through corms are rapidly expanding in areas planted in Egypt. Flower of Gladiolus is a splendor and perfection renowned as the bulbous flower's queen. Its spikes are long with huge florets, vivid variations in color, beautiful hues, varied bloom sizes, and durable vase life (Roy *et al*., 2017; Rashid, 2018). Plants of Gladiolus are now one of the most popular bulbous decorative garden plants in the world, as well as a bouquet chopped flower crop and centerpieces (Cantor and Tolety, 2011; Demir and Çelikel, 2019). Aside from their ornamental potential, their use in herbal medicine as a result of the therapeutic characteristics of the altered stems and leaves, as well as in closely related industries, boosts their significance.

Because nitrogen, phosphorous, and potassium are the fundamental constituents of proteins, nucleic acids, and physiological processes in plants, they are required in substantial quantities for gladiolus plant development, blooming, and corm formation. For optimal plant development and larger yields of high-quality blooms, gladiolus demands balanced nutrients.

As many scientists have stated, the advertisement formulation of compound 19N:19P:19K with macronutrients and micronutrients has demonstrated its advantages in terms of quality improvement of a variety of beautiful bulbs (Mansour *et al*., 2015 on *Gladiolus grandiflorus* cv. Peter Pears). The high concentration of N, P, and K in compound fertilizer may explain their powerful effects which, together with micronutrients, are necessary for plant growth and development, which are required in tiny amounts but play a crucial part in most vital plant activities.

Gladiolus flower output may be harmed by unbalanced fertilizing. Chemical fertilizers excessive use for enhancing output of crops, on the other hand, has resulted in health hazards and microbial population issues in soil, as well as being rather expensive and raising the cost of production; these considerations, taken together, stimulate the use of bio-fertilizers (Mohsin *et al*., 2015).

Due to the development of nations, the price of chemical fertilizers, and the focus on sustainable agricultural systems, interest in biological fertilizers has grown in recent years (Gewaily *et al*., 2016).

Higa (1991), University of Ryukyus, Okinawa, Japan, developed a method that separated some beneficial microorganisms which are found in agriculture soil then dubbed them effective microorganisms (EM), which were commercialized by EM Research Organization. In an EM culture, beneficial microorganisms coexist, primarily photosynthetic species of bacteria (Rhodobacter sphacrodes and Rhodopseudomonas plastris), lactobacilli (*Streptococcus lactis* and *Lactobacillus Plantarum* L. casei), yeast (*Saccharomyces* spp.), and actinomycetes (*Streptomyces* spp.), which increase photosynthesis, produce bioactive chemicals for instance, enzymes and hormones, manage soil pathogens, and speed up the breakdown of lignin materials in the soil to boost crop development and output (Higa, 2000).

There have been conflicting findings on the impact of EM on agricultural growth and production. Many researchers have observed that using EM increased crop growth and output (Javaid and Mahmood, 2010; Gewaily *et al*., 2016).

Bio-fertilizers which have various strains of microbes reduce the need of chemical fertilisers and have resulted in more quality goods without dangerous chemicals materials to protect human health in agriculture sector (Mahfouz and Sharaf-Eldin, 2007). Products which are bio-fertilizers contain cells of various living micro-organisms that can transform nutritionally significant components from inaccessible to accessible forms via biochemical procedures. Bio-fertilizing is seen as an essential component in lowering the use of synthetic fertilizers that look to be environmentally benign, boosting soil fertility, and increasing soil productivity (Gewaily *et al*., 2016).

According to certain research, using bio-fertilizers to inoculate some plants (alone or in various mixtures containing minerals fertilizers) enhanced Growth, yield, and chemical composition of plants (Singh and

Tyagi, 2019). As a result, using phosphate solubilizing microorganisms in conjunction with the use of phosphate, the issue of phosphate buildup in fields and water bodies might be resolved. Rhizobacteria known as Bacillus sp. produce metabolites like siderophores and phytohormones or solubilize minerals like phosphorus to help plant growth and they are effective root invaders (Ahmad *et al*., 2018).

Biofertilization will assist to reduce the expense of artificial fertilizers, particularly N, P, and K, as well as enhance soil fertility by preserving the soil's physical properties. Micro-organimes that are helpful and can provide nutrients from raw materials and plant wastes in the soil and make them economically and precisely available are the key components of bio-fertilizers. Microbe EM1 creates hormones found in plants, useful antioxidants and stimulating substances, that aid in the solubilization of minerals. It's been proven that using EM composting fertilizer and EM-activated liquid can enhance root development and increase germination potential and pace (Hoda, 2012).

Therefore, the primary goal of this study was to investigate the potential role of bio-fertilizers (effective microorganisms (EM1) application in combination with NPK for enhancing growth, flower output, quality, and chemical components (nutrient contents and photosynthetic pigments) of gladiolus leaves in a sustainable agricultural production system in order to reduce the amount of excessive chemical material released to the environment.

Materials and Methods

An experimental investigation was conducted outdoors in the Antoniades Botanical Garden, Horticultural Research Institute, Alexandria, Egypt. In the two seasons of 2020 and 2021, the gladiolus hybrida cultivar *"Rose Supreme"* was grown.

The medium for growing

Medium is placed in plastic pots (30 cm in diameter PVC). Each bowl holds 3.0 kg of medium. Table 1 provides the results of study the medium's physical and chemical characteristics utilized, as described by Page *et al*. (1982). The culture medium's physical components could include clay (25.7%), silt (38.7%), coarse sand (5.3%), and fine sand (30.6%).

Planting gladiolus corms

Gladiolus hybrida cv. "Rose Supreme" was planted by corms which obtained from Antoniades Botanical Garden, Horticultural Research Institute, Alex., Egypt (This variety of gladiolus was imported from Holland and the product was verified through the certificate of origin present with the importer, which was verified and formal identification of the plant material by Antoniades Botanical Garden, Horticultural Research Institute, Alex., Egypt, and there is no voucher specimen of this material has been deposited in a publicly available herbarium). Gladiolus planted in PVC pots of 30 cm diameter; corms were planted at 10 cm depth from the soil surface of plastic pots. The average circumference of the chosen corms was 8-10 cm (uniform sizes and shape). It cultivated on 20th September and 25th September of the growing seasons 2020 and 2021, respectively. The long of observation is done at 140 days after planting.

Fertilizer materials

There are 7 treatments which consist of three percent of the suggested soil amended NPK (50%NPK, 75%NPK, 100%NPK) were applied as a combined with two concentrations of EM1 (Effective Microorganisms) (1% EM1 and 2% EM1). While the control treatment was (the entire percentage of the prescribed soil amendment was applied) of 100% NPK.

The fertilizer doses and adding methods: The type of fertilizer NPK used in this experiment was chemical fertilizer (19 N: 19 P_2O_5: 19 K_2O: trace of micronutrients) was used at three doses of application. The first dose was applied of compound fertilizer started from one month after planting the bulbs at a rate of 3 g/ pot (100% NPK), whereas the rest of the doses were applied two times every three weeks at a rate

Table 1: *The primary chemical features of the growth medium.*

Growing medium	EC dSm^{-1}	pH	Anions (meq/l)			Cations (meq/l)			Available macro nutrients (ppm)		
			HCO_3	Cl	SO_4	Ca	Mg	Na	N	P	K
Clay, Peatmoss, and Sand (2:1:1 v/v/v)	2.5	7.3	3.0	19.0	3.6	10	3.0	12.1	18.5	2.0	37

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of 4 g/ pot (100% NPK) in both seasons. Calculated percent (50 and 75%) from recommended dose (100%) which equal 11gm/pot separated on three doses (3, 4 and 4 gm/pot) during growth season.

Biofertilizer (EM1): was bought from a Ministry of Agriculture and Land Reclamation, Centralized Management of Afforestation and the Environment, Egypt. EM1 was applied at the rate of 10 and 20 cm/L and it was applied three times combined with NPK. Addition of NPK fertilizer was as weight/ volume (w/ v) but biofertilizer EMI was as volume/volume (v/v). EM1 was applied at the rate of 10 and 20 cm/L dissolved with water of irrigation and then added to a pot at three times applications. All treatments were dissolved with water of irrigation and then added to a pot at three times applications during growth stages (EMI applied in the same time with NPK).

EM has been applied in a formula called EM1, Professor Higa (1991), The University of Ryukyus in Okinawa, Japan, devised a method for separating beneficial bacteria from soil and labeling them as effective micro-organisms (EM), which contain a variety of beneficial micro-organisms for example [lactic acid bacteria, photosynthetic bacteria, actinomycetes yeast and fermenting fungi].

Methodical agricultural practices such as weeding and irrigation have been implemented as basic preparation for all treatments, when necessary, as recommended.

Data recorded

Studies on the morphological and chemical components of gladiolus plants were conducted: Measurement of vegetative growth parameters were conducted at 95 days after planting but, the parameters of new corms and cormels were measurement at 140 days after planting.

Plant height (cm), number of leaves per plant, fresh and dry weights of leaves (g), leaf area $(cm)^2$, days till first floret opening (day); number of florets per spike; diameter of floret (cm); length of spike (cm); fresh and dry weights of florets per spike (g) are all indicators of vegetative growth. Additionally, the parameters of new corms and cormels were recorded, including their diameter in centimetres, weight in grammes, and quantity per plant.

Determination of certain chemical elements; using the method outlined by Moran and Porath (1980), fresh leaf samples were collected each growing season at the flower bud initiation stage to measure chlorophyll a and b in fresh leaves as (mg/g).

In accordance with the procedure provided by Cottenee *et al.* (1982), the percentages of N, P, K, Ca, and Na (%) and Fe, Mn, Zn, and Cu (ppm) in the dried leaves were estimated, the N was estimated by Kjeldal apparatus; P was measured by Spectrophotometric, While K, Na, and Ca were measured by flame photometer. Regarding determination of Fe, Mn, Zn, and Cu were measured by atomic absorption spectrometry.

Sample size was recorded in four plants in each treatment for various vegetative, floral and corm parameters. The data so collected were tabulated replication-wise on basis of four plants, and then averages were worked out.

Statistical analysis

Three replicates of the experiment were run using a completely randomised block design (CRBD) and were repeated the next year. Data was collected, statistically evaluated using the ANOVA technique, and means were compared using the "Least Significant Difference (L.S.D) test at the 5% level," as specified by Steel et al. (1997) by using MSTATC software. Statistical tests used ANOVA.

Results

The positive effect of using the biofertilizer (EM1) in combination with NPK fertilizer treatments might be attributed primarily to their simulative effect on the different vegetative growth parameters and direct and indirect role of stimulating substances (nutrients, amino acids, vitamins and plant growth regulators) which came as a result from inoculation of bacteria, all those have better effects on the plant growth, consequently improving enzymatic systems that reflect on the flowers.

Characteristics of growth

Table 2 clearly shows that all biofertilizer (EM1) treatments combined with NPK treatments had a substantial influence on the vegetative growth features measured when compared to the control (the recommended NPK fertilizer).

Table 2: *Effects of bio-fertilizer (EM1) treatment and varying rates of NPK mineral fertilization on gladiolus hybrida cv. "Rose Supreme" vegetative growth characteristics throughout the 2020 and 2021 growing seasons.*

Treatments	Plant height (cm)		Number of leaves / plant		Leaves fresh weight (g)		Leaves dry weight (g)		Leaf area (cm^2)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
1% EM1 + 50% NPK	103.56	104.96	9.30	9.60	39.40	37.51	5.50	5.31	120.45	117.97
1% EM1 + 75% NPK	118.50	116.60	10.43	10.30	44.83	42.43	6.67	6.51	141.77	139.01
1% EM1+ 100% NPK	128.50	126.60	10.53	10.73	55.73	53.61	9.03	8.85	145.79	143.45
2% EM1 + 50% NPK	108.86	111.86	10.13	9.90	45.04	43.10	6.69	6.49	136.05	134.23
2% EM1+ 75% NPK	129.96	128.23	10.60	10.50	48.13	45.45	7.63	7.44	145.39	143.30
2% EM1+ 100% NPK	139.83	142.40	10.83	11.06	57.74	55.84	9.50	9.32	145.93	143.88
Control (100% NPK)	124.16	121.86	10.46	10.60	51.33	49.63	8.90	8.62	142.85	140.74
L.S.D. $_{(0.05)}$	4.76	5.90	0.55	0.44	3.34	2.89	0.55	0.59	3.83	3.56

L.S.D. (0.05) = least significant difference at 0.05 probability level.

Plant height: As indicated in Table 2, biofertilizer (EM1) application in combination with NPK treatments had a substantial impact on plant height. The treatment with 2 percent EM1 combined with 100 percent NPK resulted in the tallest plant height in both seasons, increasing plant height by 15.67 and 20.54 percent over the control in both seasons, respectively, followed by the treatment with 2 percent EM1 combined with 75 percent NPK in both seasons. There were no statistically significant changes between plants fertilized with 1 percent EM1 mixed with 100 percent NPK and plants fertilized with only the NPK recommended dose (the control treatment).

The number of leaves per plant: Plants treated with 2% EM1 combined with 100% NPK produced the highest significant leaf number per plant values, which resulted in a 4.0 percent increase in leaf number per plant above both seasons' control treatment, as shown in Table 2. Also, it was noticed that applying any of the other bio-fertilizer treatments had similar effects on this trait with treatment with the recommended NPK fertilization, except for the treatment of 1% EM1 combined with 50% NPK.

Fresh leaves weight/plant: Table 2 shows that the nutrition by 2% EM1 in combination with 100 percent NPK as a soil treatment produced both seasons' largest fresh leaf weight. It should also be noted that There were considerable differences discovered when the plants were fertilized with 1 percent EM1 when combined with 100 percent of NPK, and both treatments yielded greater significant values than the application of 100 percent NPK alone (the control treatment).

Dry leaves weight/plant: The data revealed that plants treated with 2 percent EM1 mixed with 100 percent NPK as a soil drench had the greatest significant values of leaf dry weight per plant for both seasons (Table 2). Furthermore, no significant alterations were seen as a result of using 1% EM1 combined with 100% NPK with the previously mentioned treatment. The results revealed that treating the plants with 1 percent or 2 percent EM1 in addition to 100 percent NPK resulted in greater significant values than applying 100 percent NPK alone.

Leaf area (cm^2): Table 2 shows that for both seasons, plants fertilized with 2 percent EM1 combined with 100 percent NPK had the greatest significant values of leaf area, followed by plants treated with 1 percent EM1 combined with 100 percent NPK. Also, it was noticed that applying any of the other biofertilizer treatments had comparable effects on this characteristic with 100% NPK treatment, with the exception of fertilizing with either 1% or 2% EM1 combined with 50% NPK which resulted in lower significant values than those obtained by fertilization with 100% NPK alone. Furthermore, the data showed that plants treated with 2% EM1 in conjunction with either 75% or 100% NPK had equivalent benefits.

Characteristics of flowering

Table 3 shows that all biofertilizer applications (EM1) combined with NPK treatments had a substantial impact on the assessed characteristics of blooming when compared to the control.

Table 3: *Effect of treatment with bio-fertilizer (EM1) and different rates of the recommended NPK mineral fertilization on number of days before flowering, florets number/spike, diameter of floret, and length of spike of gladiolus hybrida cv. "Rose Supreme" throughout the two growing seasons of 2020 and 2021.*

Treatments	Number of days to flowering (day)		Number of florets/ spike		Floret diameter (cm)		Spike length (cm)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
1% (EM1) + 50% NPK	101.40	100.56	10.56	10.43	9.50	9.26	52.06	52.20
1% (EM1) + 75% NPK	98.40	97.76	11.60	11.70	10.06	10.26	67.86	70.03
1% (EM1) + 100% NPK	97.16	96.73	13.50	13.80	10.90	10.70	76.53	75.43
2% (EM1) + 50% NPK	99.46	99.23	11.23	11.50	10.56	10.26	61.36	58.86
2% (EM1) + 75% NPK	96.06	95.10	13.63	13.90	11.06	10.86	71.20	73.10
2% (EM1) + 100% NPK	95.43	94.36	14.20	14.60	11.36	11.20	78.83	77.77
Control (100% NPK)	98.06	97.86	12.26	12.03	10.26	10.66	72.06	71.53
L.S.D. $_{(0.05)}$	2.05	1.12	0.36	0.37	0.53	0.65	4.69	2.69

L.S.D. (0.05) = Least significant difference at the probability level of 0.05.

Days till the first floret opens: For the first and second seasons, according to data in Table 3, supplying plants with 2 percent EM1 and 100 percent NPK reduced the time until the first blossom appeared by 2.63 and 3.50 days, respectively, compared to the plants fertilized with 100 percent NPK alone as soil dressing. Applying 1% EM1 in combination with 75% of the recommended NPK had a comparable impact on treating the plants with 100% of the recommended NPK alone. It was also found that treating the plants with 2% EM1 in combination with 75% or 100% of the required NPK had comparable effects on this characteristic.

Florets number per spike: Table 3 demonstrates that using 2% EM1 mixed with 100% NPK as a soil treatment that can be used in the two seasons resulted in the most levels of significance for florets per spike. The number of florets increased by 1.94 and 2.57 in both seasons, respectively, when the plants were fertilised with 100% NPK alone as soil dressing.

There were no significant differences between using 2 percent EM1 combined with 75 percent NPK or using 1 percent EM1 paired with 100 percent NPK to fertilize the plants. The last-mentioned treatments gave superior significant values to those obtained as a result of applying the 100% NPK alone.

Diameter of the floret (cm): Table 3 shows that using 2 percent EM1 in combination with 100 percent NPK resulted in the largest significant increases in floret diameter, with increases of 1.1 and 0.54 cm for both seasons, respectively, in comparison to plants that only received the 100 percent recommended NPK.

Length of a spike (cm): The maximum spike length values were observed when 2 percent EM1 was applied in conjunction with 100 percent NPK for both seasons, as shown in Table 3. When compared to fertilising the plants with 100% NPK alone, this treatment resulted in length of spike increases of 6.77 cm and 6.24 cm for the both seasons, respectively. Additionally, no discernible alterations were seen when the aforementioned treatment was applied, and the plants were fertilised with 100% NPK and 1% EM1. Additionally, the data showed that fertilising the plants with 1% EM1 in addition to 75% NPK had effects with this feature that were comparable to those achieved by using 100% NPK alone.

Fresh weight of florets per spike (g): The most for both seasons, using 2% EM1 combined with 100% NPK resulted in substantial values of florets per spike fresh weight, as displayed in Table 4. Furthermore, the findings showed that application of 2 percent EM1 in combination with 75 percent NPK had similar impacts on this characteristic as applying 100 percent NPK alone.

Dry weight of florets per spike (g): Using 2% EM1 in conjunction with 100%percent NPK for both seasons resulted in the highest recorded values of florets per spike dry weight, as shown in Table 4. Moreover, the results proved that applying 2% EM1 combined with 75% NPK gave the same effects as applying 100% NPK alone.

Flowering duration (day): Table 4 shows that application of 2 percent EM1 in conjunction with 100 percent NPK with an increment of 1.36 and 0.76 days for the two seasons, respectively, resulted in highest values ever recorded of flowering duration when compared to fertilizing the plants with 100 percent NPK applied alone.

Characteristics of corm

Table 4 shows that, when compared to the control, the analyzed parameters of corms appeared to be considerably changed as a result of applying biofertilizer combined with NPK treatments (100% of the recommended NPK applied alone).

Diameter of new corm (cm): The greatest significant values of new corm diameter were recorded when 2 percent EM1 was paired with 100 percent NPK for both seasons, as displayed in Table 4. Also, the data demonstrated that the previously mentioned treatment, 2% EM1 paired with 75% NPK, had a similar effect on this feature. Moreover, both of those treatments gave significantly superior values than those obtained after applying 100% NPK alone, in the two seasons.

Fresh weight of new corm (g): The highest significant values of new corm fresh weight were obtained for both seasons when the plants were treated with 2 percent EM1 and 100 percent NPK, followed by a treatment with 2 percent EM1 mixed with 75 percent NPK yielded the highest significant values of new corm fresh weight (Table 4). The previously mentioned treatments gave significantly higher values than those obtained as a result of applying 100% NPK alone, for both seasons.

Photosynthetic pigments in leaves

As demonstrated in Table 5, leaf chlorophyll content (a and b) appeared to be considerably changed by using biofertilizer (EM1) in combination with NPK treatments when comparison to the control.

Leaf chlorophyll content (mg/g L.F.W): Chlorophyll (a) concentrations were highest when the plants were fertilized with 2 percent EM1 combined with 100 percent NPK, followed by a treatment of 1 percent EM1 mixed with 100 percent NPK (Table 5). Furthermore, the previously described treatments yielded much higher values than those obtained after treating the plants with 100% NPK.

The greatest significant chlorophyll (b) values were obtained after fertilizing the plants with 2% EM1 mixed with 100% NPK, followed by a treatment of 2% EM1 mixed with 75% NPK., for both seasons, according to the results shown in Table 5. The impact of the previously described treatment on this feature was identical to that achieved by fertilizing the plants with 100 percent NPK supplied alone.

Gladiolus leaves nutrient content

The findings in Tables 5 and 6 show that biofertilizer application (EM1) combined with NPK treatments had a substantial effect on the nutrient contents when comparison towards the control.

Table 4: *Effect of treatment with bio-fertilizer (EM1) and different rates of the recommended NPK mineral fertilization on fresh and dry weights of florets per spike, flowering duration, new corm diameter, and fresh weight of corm of gladiolus hybrida cv. "Rose Supreme" throughout the 2020 and 2021 growing seasons.*

Treatments	Floret fresh weight /spike		Florets dry weight/spike		New corm fresh weight		New corm diameter		Flowering duration	
	(g)						(cm)		(day)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
1% (EM1) + 50% NPK	20.26	20.73	2.34	2.21	21.39	21.16	3.26	3.15	9.53	9.30
1% (EM1) + 75% NPK	30.06	30.99	4.2	4.08	25.44	25.22	3.51	3.38	11.16	11.23
1% (EM1) + 100% NPK	31.27	32.09	4.62	4.45	30.14	29.86	4.45	4.31	12.40	12.26
2% (EM1) + 50% NPK	27.73	28.16	3.94	3.86	28.31	28.03	4.20	4.05	11.56	11.36
2% (EM1) + 75% NPK	31.83	31.96	4.73	4.56	34.32	34.04	4.63	4.52	12.26	12.23
2% (EM1) + 100% NPK	32.64	32.87	5.23	5.11	35.94	35.32	4.78	4.57	12.96	12.66
Control (100% NPK)	30.35	30.67	4.75	4.66	30.50	30.21	4.24	4.13	11.60	11.90
$L.S.D._{(0.05)}$	1.88	1.89	0.29	0.31	0.47	0.57	0.18	0.15	0.55	0.58

L.S.D. (0.05) = Least significant difference at the probability level of 0.05.

Table 5: *Effect of treatment with bio-fertilizer (EM1) and different rates of the recommended NPK mineral fertilization on photosynthetic pigment contents (chlorophyll a& b, mg/g leaf fresh weight) and leaf NPK contents (%) of Gladiolus hybrida cv. "Rose Supreme" during the 2020 and 2021 growth seasons.*

Treatments	Chlorophyll (a)		Chlorophyll (b)		N		P		K	
	(mg/g leaves fresh weight)				(%)					
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
1% (EM1)+50% NPK	1.35	1.39	7.06	7.22	1.06	1.07	0.162	0.164	3.50	3.60
1% (EM1)+75% NPK	2.53	2.64	9.01	9.63	1.21	1.21	0.179	0.183	3.73	3.83
1% (EM1)+100% NPK	4.56	4.35	12.57	12.81	1.27	1.29	0.201	0.208	3.67	3.86
2% (EM1)+50% NPK	2.44	2.27	10.20	10.43	1.14	1.15	0.164	0.168	3.53	3.63
2% (EM1)+75% NPK	4.46	4.24	13.09	13.40	1.28	1.29	0.204	0.211	3.93	4.10
2% (EM1)+100% NPK	5.67	5.48	14.22	14.49	1.41	1.44	0.210	0.216	4.06	4.16
Control (100% NPK)	4.19	4.16	12.50	12.35	1.20	1.23	0.169	0.173	3.73	3.83
L.S.D.$_{(0.05)}$	0.17	0.18	0.79	0.76	0.011	0.029	0.005	0.007	0.136	0.120

L.S.D. (0.05) = Least significant difference at the probability level of 0.05.

Content of nitrogen, phosphorus, and potassium (%): Table 5 shows that plant leaf assays for the content of N, P, and K (percentage). The findings demonstrated that using a biofertilizer in combination with NPK had a substantial impact on leaf NPK content. The most significant values of leaf N content for both seasons were obtained by feeding the plants with 2 percent EM1 mixed with 100 percent NPK, followed by fertilizing the plants with 2 percent EM1 combined with 75 percent NPK. The results of the treatments outlined above were more substantial than those achieved by fertilizing the plants with 100 percent of the NPK. Also, for both seasons, no significant changes were seen between the treatments of applying 2 percent EM1 mixed with 75 percent NPK and fertilizing the plants with 1 percent EM1 mixed with 100 percent NPK. Furthermore, the results showed that feeding the plants with 1 percent EM1 in combination with 75 percent NPK had similar effects as fertilizing the plants with 100 percent NPK alone.

The data indicated that the most significant levels of leaf P content were obtained when fertilising the plants with 2 percent EM1 in addition to 100 percent NPK, followed by fertilizing the plants with 2 percent EM1 combined with 75 percent NPK, and both of these treatments gave higher significant values of leaf P content than those obtained after fertilizing the plants with 100 percent NPK. It was also found that treating the plants with 2 percent EM1 in combination with 50 percent NPK produced similar effects to fertilizing the plants with 100 percent NPK alone.

In terms of the effect of biofertilizer application combined with NPK on leaf K content, the results showed that all Applications of biofertilizers generated more significant results than those attained after solely fertilising the plants with 100% NPK, except for the treatment of applying 2 percent EM1 combined with 50 percent NPK, which produced similar results. The findings showed that the highest significant values of leaf K content were obtained when the plants were treated with 2 percent EM1 together with 100 percent NPK and that this treatment produced equivalent results to treating the plants with 2 percent EM1 mixed with 75 percent NPK during both seasons. Furthermore, administering 1% EM1 in combination with 75% NPK produced results that were comparable to those achieved after fertilizing the plants with 100% NPK administered alone.

Leaf calcium and sodium contents (%): When it came to the effects of biofertilizer application combined with NPK on a leaf Calcium content, the data showed in Table 6 revealed that using 2% EM1 combined with 100% NPK gave the highest significant values of this trait, with no significant differences when compared to the treatment of using 2% EM1 combined with 75% NPK. It should also be noted that fertilizing the plants with 1% EM1 in combination with either 75 percent or 100 percent NPK produced identical results.

Table 6: *Effect of treatment with bio-fertilizer (EM1) and different rates of the recommended NPK mineral fertilization on Gladiolus hybrida cv. "Rose Supreme" leaf Na and Ca (%) and leaf Fe, Mn, Zn, and Cu contents (ppm) over the two growth seasons of 2020 and 2021.*

Treatments	Na		Ca		Fe		Mn		Zn		Cu	
	(%)				(ppm)							
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
1% (EM1) + 50% NPK	0.29	0.30	1.73	1.83	96	97	23	24	53	55	2	3
1% (EM1) + 75% NPK	0.41	0.42	1.86	1.96	133	135	25	26	55	57	4	5
1% (EM1) + 100% NPK	0.32	0.33	1.90	2.00	138	139	27	27	58	60	5	6
2% (EM1) + 50% NPK	0.40	0.41	1.86	1.96	99	101	24	24	55	56	3	4
2% (EM1) + 75% NPK	0.43	0.43	2.10	2.20	139	142	29	29	62	63	6	7
2% (EM1) + 100% NPK	0.43	0.43	2.13	2.23	165	167	30	31	67	68	7	8
Control (100% NPK)	0.33	0.34	1.86	1.96	120	121	25	26	55	56	3	4
L.S.D. $_{(0.05)}$	0.01	0.01	0.08	0.18	2.52	3.16	1.02	1.09	1.24	1.14	0.67	1.31

L.S.D. (0.05) = Least significant difference at the probability level of 0.05.

The results listed in Table 6 demonstrated that applying 2 percent EM1 along with 75 percent NPK to the plants produced the most significant levels of leaf sodium content. For both seasons, no significant changes were seen when 2 percent EM1 was used in combination with 75 percent or 100 percent NPK. When 1 percent EM1 was coupled with 50 percent NPK, the least significant values of leaf Na content were detected.

Leaf Fe, Mn, Zn, and Cu contents (ppm): Table 6 shows that all of these factors had a substantial impact on leaf Fe, Mn, and Zn content (ppm). The greatest significant levels of these elements were found after fertilize the plants with 2% EM1 mixed with 100% NPK, followed by 2% EM1 mixed with 75% NPK, for both seasons. It's also worth noting that all biofertilizers coupled with NPK resulted in higher significant values of leaf Fe content than those obtained after feeding the plants with 100 percent NPK alone. Furthermore, the findings demonstrated that treating the plants with 1 percent EM1 in combination with 75 percent NPK had similar effects on leaf Mn content as applying 100 percent NPK alone.

Table 6 shows that treating the plants with 2 percent EM1 paired with 100 percent NPK provided the most significant values of this characteristic in the two seasons, no significant changes were seen when 2 percent EM1 was paired with either 100 percent or 75 percent NPK. It was also found that using 1% EM1 in combination with either 100% or 75% NPK had similar results.

Discussion

Data illustrated in the results appeared that all biofertilizer (EM1) applications combined with NPK treatments had a considerable impact on the investigated vegetative growth metrics, flower and corm characteristics, Photosynthetic pigments in leaves, and nutrient content compared to the control (the recommended NPK fertilizer).

As many experts have stated, employing compound fertilizer 19N:19P:19K has proven to be superior for boosting the quality of many ornamental bulbs (Mansour *et al*., 2015 on Gladiolus grandifloras cv. Peter Pears). The powerful activity of compound 19N:19P:19K may be attributed to its high levels of nitrogen, phosphorus, and potassium, all of which are necessary for plant growth and development. Micronutrients, on the other hand, are essential for the majority of vital plant processes, despite the fact that they are only required in minute amounts.

These effects might also be ascribed to the EM1 application's impact on soil pH reduction, soil organic matter increasing, soil water and nutrient uptake, and improving soil fertility. Furthermore, the amount of soil microflora, including total bacteria, actinomyces, and fungus precursors of gibberellins and indole acetic acid was increased by the EM1 treatment, resulting in improved root system development and improved nutrient absorption, resulting in improved plant health (Ibrahim, 2012).

In plants, the EM1 causes photosynthetic processes to be activated (which increases the production of chlorophyll, protein and a variety of enzymes, including peroxidase activity) (Winget and Gold, 2007). EM1 has the ability to boost the production of chlorophyll, a green pigment that aids in the carbon dioxide, solar energy, and other chemicals absorption, as well as plant growth and development. The impact of EM on growth of root, followed by greater nutrient nurturing, might explain the increased leaf area. This means that biomass output and photosynthetic capability have increased. According to Fikry *et al.* (2020), EM includes phytohormones or other chemicals that are active physiologically postpone plant senescence and improve photosynthetic activity.

According to Olle and Williams (2013), the positive impact of biofertilizer in this regard can be due to its influence on accelerating nitrogen fixation, increasing the formation of chemicals that promote growth, or producing organic acids, and the augmentation of nutrient absorption. Furthermore, Fikry *et al.* (2020) suggests that the capacity of these organisms to produce growth regulators that control nutrient uptake and the formation of root biomass, such as auxins, cytokinins, and gibberellins, could explain the increase in plant growth caused by N-fixed bacteria inoculation.

Strong growth and a high yield of high-quality plants were produced by treatments using the bio-fertilizer (EM1) in conjunction with dosages of 50, 75, and 100% of the necessary N, P, and K from the same nutrient that is used in soil application. Additionally, the value or superiority of bio fertiliser applications was considered when determining whether to use costly mineral-N, P, and K fertilisers or increase the income from gladiolus crops. It was also considered when determining how to reduce the potential negative health and environmental effects of the unrestrained use of mineral-N fertilisers (Hellal *et al.*, 2011).

Increases in each of the growth characteristics (plant height, etc.) were caused by a variety of factors, including: Its ability to release molecules that may have aided plant growth, specifically gibberellic, IAA acid and cytokinin-like substances; the production of some vitamins, such as B12; the increased uptake of water and minerals from the soil, an expansion of the root's surface area, the number of its hairs, and its length may be to blame for this; increased output of biologically active fungistatical compounds, which may alter the microflora in the rhizosphere and affect the balance of dangerous and helpful species; and Increased ability to convert N2 to NH4 and thereby make it available to the plant. The improvement in the observed growth parameters may be linked to bio-fertilizers' positive impact on the availability and absorption of nutrients (Ghabour *et al.*, 2019).

Many researchers have shown how bio fertilizers help to lower pH by organic acids such as acetic, propionic, fumaric, and succinic acids which are secreted, which help to dissolve nutrients linked to organic materials and make them accessible to developing plants. The beneficial effects of bio-fertilizers had a significant impact on plant development, which resulted in increased output. This rise might be attributed to the action of N generated by bacteria species, as well as growth regulators such as IAA and GA3 that encouraged growth (Itelima *et al.*, 2018).

Rhizobacteria called Plant Growth Promoting Rhizobacteria (PGPR) aid in the growth of plants. Microorganisms can stimulate us in two ways: directly or indirectly. Direct impacts include phytohormone synthesis, increased mineral availability, phosphate and micronutrient release, nonsymbiotic nitrogen fixation, and promotion of disease-resistance mechanisms. The alteration of the root environment and ecology by (PGPR) has indirect consequences. Acting as biocontrol agents and preventing disease transmission, for example, or releasing antibiotic compounds that destroy unpleasant microorganisms. It has been postulated that adding biofertilizer increases plants' ability to convert N2 to NH4 and so makes it available to them. Bio-fertilizers (EM1) that provide 50 to 100 percent of the required N, P, and K doses from the same nutrient as a soil application, on the other hand, resulted in strong growth and excellent yield of Gladiolus plants with good quality (Ghabour *et al.*, 2019; Itelima *et al.*, 2018).

Furthermore, the ability of phosphate-dissolving bacteria to encourage plants to produce growth-promoting compounds like auxine, gibberellins, and cytokinens, which may enhance plant growth and stimulate microbial development in the rhizosphere, may be the cause of the beneficial effect these bacteria have on growth, as well as their positive effect on organic and inorganic phosphorus mineralization and solubilization potential. Siderophores, hydrocyanic acid (HCN), IAA, and other associated activities such as excellent phosphate solubilization, competition in soil, and root colonisation are all produced are well known to be attributed to the beneficial effects of plant growth-promoting rhizobacteria. Furthermore, it was shown that using phosphate-dissolving bacteria as a biofertilizer reduced soil pH, increasing the solubility of several minerals including P, Fe, Zn, Mn, and Cu, and therefore increasing nutrient absorption by plants (Fikry *et al*., 2020).

The observed effects and determine the overall factors affecting the efficiency of biofertilizers are many, the most important of which are the C/ N ratio, ventilation, soil moisture content, and degree of reaction PH of the soil and maintaining long-term soil fertility.

Due to rising fertilizer prices and soil and water contamination, improving nitrogen, phosphorous, and potassium usage efficiency is a financial and environmental need. The current fertilizer price increase is one of the major roadblocks to increasing crop economic production. As a result, efforts must be made to reduce its losses and maximize its economic value. This bio-fertilizer (EM1) resulted in increased N, P, and K available for Gladiolus plants to absorb, leading to an increase in measurable growth characteristics (plant height, etc.).

The aforementioned findings so demonstrated that it was possible to achieve the best output by saving approximately 50 or 25 percent of mineral N, P, and K (through the application of bio-fertilizer combined with NPK treatments) and avoiding its undesirable effects.

Conclusions and Recommendation

There are more opportunities now for the production and use of biofertilizer products in agriculture as a substitute strategy due to the pressing need to achieve sustainable agricultural goals, decreasing dependence on agrochemicals, and growing consumer preference for more nutrient-dense foods. The current study found that using a bio-fertilizer (EM1) in combination with NPK treatments considerably raised growth and yield, as well as improved quality since it enhances bio-N, P, and K production while reducing mineral usage of these nutrients by 25 to 50%. This improvement might be due to a direct effect of chemical fertilizers or an indirect effect from the stimulation of microbial multiplication. Furthermore, as eco-friendly and cost-effective inputs for farmers, bio-fertilizer would play a major role in soil production and sustainability, as well as in environmental protection. However, proper application of biofertilizers depends on understanding of the production process, application strategies, and promotion of biofertilizers. Therefore, additional research is required to increase the effectiveness of this approach.

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Novelty Statement

Incorporation of nutritional sources (bio or in-organic fertilizers) either applied alone or in combination boosted the growth and quality parameters of Gladiolus Hybrida cv. Rose Supreme.

Author’s Contribution

Sona S. El-Nwehy: Suggested and put the idea and was a major contributor in writing the manuscript.
Assem A. M. El-Naggar: Executed the experiment and taking vegetative measurements.
Adel B. El-Nasharty: Performed chemical analyzes and statistical analysis of data. All authors read and approved the final manuscript.

List of abbreviations

EM: effective microorganisms; NPK: Nitrogen, Phosphorus, Potassium

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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