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RESPONSE OF TUBEROSE PLANTS (*POLIANTHES TUBEROSA* L.) TO CHEMICAL AND BIO FERTILIZATION AND THEIR EFFECT ON VEGETATIVE GROWTH, FLOWERING AND CHEMICAL COMPOSITION UNDER SANDY SOIL CONDITIONS

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ABSTRACT: A field experiment was carried out at the Experimental Farm of El-Kassasin Horticultural Research Station, Agricultural Research Center, Ismailia Governorate, Egypt, to study the effect of partial substitution of chemical nitrogen fertilizer with biofertilizer containing two strains of nitrogen-fixing (*Azospirillum lipoferum, Azotobacter chrococcoum*), on vegetative growth, flowering and chemical composition of tuberose plants (*Polianthes tuberosa* L.) under sandy soil conditions to rationalize the use of mineral fertilizers and reduce pollution of the environment in order to increase the quantity and improvement quality of the product on the tuberose plants during the two successive seasons of 2017 and 2018. This experiment included seven treatments with three replications, which were the combinations between two of mineral nitrogen rates (50% and 75%) and three bio-fertilizers of nitrogen-fixing bacteria (*Azospirillum lipoferum* (S), *Azotobacter chrococcoum* (Z) and *Azospirillum lipoferum* + *Azotobacter chrococcoum* (S*Z) treatments in addition to the control which was 100% N. The treatments were arranged in Randomized Complete Block Design. The results showed that fertilizing tuberose plants with mineral nitrogen at 100% of the recommended dose recorded the highest values for plant height, number of leaves/plant and number of florets/spike. while fertilization with mineral nitrogen at 75% plus the dual inoculation with *Azospirillum* + *Azotobacter* recorded the highest values for fresh and dry weights of leaves (g)/plant, fresh and dry weights of root (g)/plant, fresh and dry weights of spike (g)/plant, floret diameter (cm), spike length (cm), longevity of tuberose cut flower spikes (days), in addition to leaves content of N, P, K, total chlorophyll a, b and carbohydrates %, as well as essential oil percentage in flowers without significant difference between them and control in most cases in both seasons.

Key words: Tuberose, Spike, Nitrogen, Bio-fertilizers, *Azospirillum* and *Azotobacter*.

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INTRODUCTION

Polianthes tuberosa L. is a perennial bulbous plant of the *Agavaceae* family, and is an ornamental bulb plant native to Mexico (Benschop, 1993). It has a good economic potential for cut flower trade and essential oil industry (Alan *et al*., 2007). Tuberose is one of the common cultivated flowering bulbs used as garden plant with a great demand for its attractive tall spikes with fragrant cut flowers and also for extraction of its highly valued natural flower essential oils (Jawaharlal *et al*., 2006). In France and India, tuberose plant was widely cultivated as a source of essential oils for the perfume industry (Majid *et al*., 2012).

Tuberose also responses well to the application of mineral fertilizers, which are observed to be varying depending on the climatic condition and soil types. Nitrogen, phosphorus and potassium markedly influence the growth and development of plants. However, reliance on chemical fertilizers increases soil and environmental pollution (Qureshi, 2007). Patel (2005) stated that, application of nitrogen at 400 kg/ha on tuberose significantly recorded the highest values of vegetative, floral and quality characters viz., plant height, number of leaves, number of days to first flowering, spike length, number of florets/spike and diameter of floret. Also, Khalaj *et al.* (2012) showed that using 200 kg/ha N can improve growth and yield characters of tuberose as flower stalk height, stem diameter, bulb weight, chemical constituents (chlorophyll a, b and carotenoids contents and N, P, K percentages of leaves. Recently, Kumar *et al.* (2017, whereas, maximum plant height, number of leaves, fresh and dry weights of leaves, spike length, rachis length, number of florets per spike and vase life was recorded with N_2 (60 kg/acre nitrogen). In this regard, the maximum plant height and number of leaves per clump of tuberose were observed by nitrogen at 20 g/m^2 (Nain *et al.*, 2018).

In the recent years, the safe agriculture is one of the main attitudes in the world (El-Kouny, 2002). Bio-fertilizers are reasonably safer to the environment than chemical fertilizers and play an important role in decreasing the use of chemical fertilizers. Consequently, it causes a reduction in environmental pollution. Soil inoculation with micro-organisms lead to increase soil available nitrogen and consequently increase formation of metabolites which encourage the plant vegetative growth and enhance the meristematic activity of tissues to produce more branches. Also, N-fixers synthesize stimulatory compounds such as gibberellins, cytokinins and IAA that act as growth regulators (Sperenat, 1990 and Dadarwal *et al*., 1997). Barman *et al*. (2003) studied the effect of biofertilizers, alone or with other fertilizers, on the growth and yield of tuberose (*Polianthes tuberosa*) results showed that, plant height and spike emergence were significantly enhanced with *Bacillus firmus* inoculation compared to the untreated control. Attia *et al*. (2005) studied the response of chemical constituents of *Gladiolus grandiflorus*, L. "Eurovision" to different N-fixing bacteria treatments, they found that chemical constituents (chlorophyll a, b and carotenoids contents, N, P, K percentages of leaves and corms and reducing sugars content in the leaves and corms) were significantly augmented as a result of using the different N-fixing bacteria treatments, and the most effective treatment in this respect was the dual strain (*Azotobacter chroococcum* and *Azospirillum brasilense*). Kandeel *et al*. (2002) on *Ocimum basilicum* L. plant found that dual inoculation with symbiotic N_2 fixers (*Azotobacter* and *Azospirillum*) with half or full doses of inorganic N fertilizer increased plant height, number of branches per plant, and fresh and dry weights of leaves as well as roots. Also Ali *et al*. (2014) reported that application of biofertilizers to gladiolus showed that all vegetative and reproductive growth accomplished successfully, however, the treatment containg *Azospirillum* gained highest values in terms of plant height, florets spike, spike length, florets fresh weight and vase life.

Therefore the aim of the present study was to clarify the effect of partial substitution of chemical nitrogen fertilizer by inoculation with *Azospirillum lipoferum* and *Azotobacter chrococcoum* on growth, flowering and chemical composition of tuberose plants under sandy soil conditions to rationalize the use of mineral fertilizers and reduce pollution of the environment in order to increase the quantity and to improve quality of the product of tuberose plants.

MATERIALS AND METHODS

Afield experiment was carried out at the Experimental Farm of El-Kassasin Horticultural Research Station, Ismailia Governorate, Egypt, during the two successive seasons of 2017 and 2018.

Plant material:

Bulbs of tuberose with 30 – 35 mm diameter (about 25 - 35 g weight) were obtained from a private farm at El Qanatir El Khaireya, Qualubya Governorate, Egypt.

The physical and chemical properties of the experimental soil used in this experiment are showen in Table (1). While the dry compost was added to the experimental area during preparing soil before planting in the two seasons as a rate of 10 tons/fed and the analysis of the used compost was recorded in Table (2).

Table 1. The physical and chemical properties of the experimental soil in the two seasons.

Physical characteristics		Chemical characteristics	
Field capacity	11.77	$CaCO_3$ %	12.25
Available water	7.57	Organic matter %	0.08
Wilting point	4.20	pH	7.5
Coarse sand	73.08	EC (ds/m)	2.14
Fine sand	15.7	Ca (mg/100g)	0.14
Silt	7.2	Na (mg/100g)	0.34
Clay	4.02	K (mg/100g)	0.16
Texture class	Sandy	Cl (mg/100g)	0.30

Table 2. Compost analysis.

Fresh weight	750 – 850 kg	**Macro elements (%)**	
Dry weight (m^3)	550 – 650 kg	N %	0.8 – 1.2
Moisture %	25 – 30 %	P %	0.4 – 0.6
pH	7.5 – 8.5	K %	0.8 – 1.4
EC ds/m	3.5 – 6.5	**Micro elements (ppm)**	
Saturation %	150 – 200	Fe	1000 – 1800
Organic matter %	25 – 30	Mn	80 – 120
Organic carbon	14.5 – 17.5	Cu	100 – 160
C/N ratio	1:14.5 or 1:18	Zn	30 – 50

Chemical nitrogen fertilizer:

The nitrogen fertilizer used was ammonium nitrate 33.5% N, and the recommended dose of this fertilizer was 80 kg N/fed. The treatments of this factor were [100% as a control, 75% (60 Kg N/fed) and 50% (40 kg/fed) from the recommended dose]. Nitrogen fertilizer was divided to four equal portions and was added after 30, 45, 60 and 75 days from sowing.

Microorganisms:

Microorganisms bacteria used [*Azospirillum lipoferum* (S) and *Azotobacter chrococcoum* (Z)] were obtained from Microbiology Department, Soil Water and Environment Research Institute, Agric. Res. Center (ARC), Giza, Egypt. The N_2-fixers inoculum (1 kg/fed) was mixed with tuberose bulbs by adding Arabic gum solution, then bulbs coated with the inoculum before sowing and the treated bulbs were directly sown in the same day.

The arrangement of treatments:

This experiment included seven treatments with three replications, which were the combinations between two of mineral nitrogen rates (50% and 75%) and three bio-fertilizers of nitrogen-fixing bacteria (S, Z and S*Z) treatments in addition to the control which was 100% N. The treatments were arranged in Randomized Complete Block Design as simple experiment, as follow:

1. 50 % N + *Azospirillum lipoferum* (S).
2. 50 % N + *Azotobacter chrococcoum* (Z).
3. 50 % N + *Azospirillum lipoferum* + *Azotobacter chrococcoum* (S*Z).
4. 75 % N + *Azospirillum lipoferum* (S).
5. 75 % N + *Azotobacter chrococcoum* (Z).
6. 75 % N + *Azospirillum lipoferum* + *Azotobacter chrococcoum* (S*Z).
7. Control (100% N).

Cultivation procedures:

The bulbs were planted in the 10th April, for the two seasons. The bulbs were planted on rows, at 30 cm between plants in the row (23300 bulbs per feddan). The experimental unit area was 9 m^2 it contained three rows with 5 m in length and 60 cm in width.

Harvesting stage:

The spikes were harvested when 2-3 pairs of florets from the bottom of the spike opened. The individual spike was harvested by cutting it from the bottom.

Holding solution treatment:

From all treatments tuberose cut flower spikes were held till the end of the experiment in holding solution which was composed of sucrose (S) + 8–hydroxyl–quinoline sulphate (8–HQS) + citric acid (CA) [S + 8–HQS + CA] : at 4%, 400 ppm and 100 ppm concentrations, respectively. The pH of holding solution was 3.45.

Data recorded:

The following characters were recorded:

Vegetative characters:

Records were taken at flowering stage as for:

1. Plant height (cm).
2. Number of leaves/plant.
3. Fresh and dry weights of leaves (g)/plant.
4. Fresh and dry weights of root (g)/plant.

Floral characters:

1. Number of florets/spike.
2. Floret diameter (cm) in tuberose spike from 2 pair of florets from the bottom of the spike opened.
3. Fresh and dry weights of spike (g)/plant.
4. Spike length (cm).

Postharvest characters:

Longevity of tuberose cut flower spikes (days) was determined when the wilted florets reach 75% from the number of the total florets on the spikes according to El-Saka (1992) on tuberose cut flower spikes.

Chemical characters:

1. Total nitrogen, phosphorus and potassium percentages in leaves were determined according to the procedure described by Mazumder and Majumder (2003).
2. Chlorophyll a and b: fresh leaf samples were taken for measuring the chlorophyll a and b according to Mazumder and Majumder (2003).
3. Total carbohydrates % (d.w.) were determined according to Herbert *et al.* (2005).
4. Essential oil percentage extraction: Hexane was used as solvent to extract the scents from tuberose flowers because it is strongly non-polar solvent and is frequently used insolvent to extract oils. 100 g of flowers were soaked in 1 liter of solvent for 1 h. After removing the debris, the solvent was evaporated leaving the concrete behind. Tuberose absolute was extracted from concrete sample using alcohol according to Rakthaworn *et al.* (2009).

Statistical analysis:

The statistical analysis of the present data was carried out according to Steel and Torrie (1980) using L.S.D. at 5% and 1% levels for comparison between means of the different treatments.

RESULTS AND DISCUSSION

Vegetative growth:
1. Plant height:

Data presented in Table (3) show the effect of nitrogen fertilization rates and biofertilizers on plant height of tuberose. It is obvious from the data that, fertilizing tuberose plant with 100% mineral nitrogen (control) significantly was the superior treatment for increasing plant height followed by fertilization with mineral nitrogen at 75% plus the dual inoculation

Table 3. Effect of nitrogen fertilizer rates and bio-fertilizer kinds on plant height and number of leaves/plant of tuberose plants during the two seasons.

	Plant height (cm)		No. of leaves/plant	
	1st season	2nd season	1st season	2nd season
50% N + S	55.3	59.3	32.7	33.7
50% N + Z	61.3	63.7	34.3	38.0
50% N + S*Z	65.3	68.3	37.3	41.3
75% N + S	72.7	73.7	55.7	59.7
75% N + Z	74.3	75.3	57.7	62.0
75% N + S*Z	81.0	82.3	58.7	64.3
100% N	81.3	82.0	59.7	66.3
LSD. at 5%	1.1	0.8	1.7	1.9
LSD. at 1%	1.5	1.1	2.4	2.7

N= nitrogen, S= *Azospirillum lipoferum*, Z= *Azotobacter chrococcoum* and S*Z= *Azospirillum lipoferum* + *Azotobacter chrococcoum.*

Azospirillum + *Azotobacter* without significant differences between them. On the contrary, the lowest values in this connection were reported generally in case of 50% mineral nitrogen plus inoculation with *Asospirillum* in both seasons.

The increment in plant height due to the application of high rate mineral N (80 kg N/fed) may be attributed to the pronounced role of nitrogen in plant metabolism. Nitrogen is a constituent of proteins, enzymes, hormones, vitamins, chlorophyll and photosynthesis which led to an increase in plant metabolism and vegetative growth (Reddy and Reddi, 2002), while the favorable effect of biofertilizer on vegetative growth could be attribute to the activity of bacteria in the absorption zone of plant root by improving soil fertility and consequently plant development by N_2-fixation and due to releasing of certain other nutrients; i.e., Fe, Zn, and Mn (Bhonde *et al*., 1997) through the breakdown of organic materials in the soil and make these elements in available forms.

2. Number of leaves/plant:

Regarding the effect of mineral nitrogen fertilization rates and biofertilizers on number of leaves of tuberose plants, the data illustrated in Table (3) reveal that, the highest value recorded with the treatment of 100% mineral nitrogen followed by mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter* with no significant difference between them in the first season and with a significant difference in the second one. Furthermore, the differences between the combined treatment of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter* and other treatments were highly significant.

Similarly, the effect of nitrogen fertilizer on number of leaves of tuberose was studied by (Nain *et al*., 2018). While Increasing plant growth due to free living N fixating bacteria such as *Azotobacter, Azospirillum, Bacillus polymixa, Enterobacter* and *Klebsiela* were found to have not only the ability to fix nitrogen but also to release certain phytohormon of gibberlic and indolic nature compounds which could stimulate plant growth, absorption of nutrients and photosynthesis process (Fayez *et al*., 1985).

3. Fresh weight of leaves (g)/plant:

Data in Table (4) show the effect of mineral nitrogen fertilization rates and biofertilizers on fresh weight of leaves/plant of tuberose. The treatments had clear influences on the fresh weight. The differences between majority of treatments were highly significant. The superior effect obtained from the combined treatment of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter,* followed by control (100% N), with highly significant effects during both seasons. The aforementioned results also were in harmony with those obtained by Patel (2005) on tuberose and Attia *et al*. (2005) on *Gladiolus grandiflorus*.

Table 4. Effect of nitrogen fertilizer rates and bio-fertilizer kinds of fresh and dry weights of leaves and roots (g)/plant on tuberose plants during the two seasons.

	Fresh weight of leaves (g)/plant		Dry weight of leaves (g)/plant		Fresh weight of root (g)/plant		Dry weight of root (g)/plant	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
50% N + S	24.7	25.7	4.37	4.00	51.3	53.0	10.9	11.9
50% N + Z	26.3	29.3	4.77	4.73	55.0	60.0	12.2	12.7
50% N + S*Z	29.7	34.3	5.23	5.80	66.0	70.3	15.3	15.6
75% N + S	32.3	36.3	5.83	6.43	77.7	82.3	17.3	18.4
75% N + Z	36.3	40.7	6.23	7.07	85.3	89.0	18.7	19.8
75% N + S*Z	43.7	46.7	6.70	7.93	93.7	99.7	20.4	22.0
100% N	39.0	41.3	5.97	6.90	92.7	96.0	20.2	21.2
LSD. at 5%	1.5	1.1	0.18	0.22	1.3	2.1	0.3	0.4
LSD. at 1%	2.1	1.6	0.25	0.31	1.9	2.9	0.5	0.6

N= nitrogen, S= *Azospirillum lipoferum*, Z= *Azotobacter chrococcoum* and S*Z= *Azospirillum lipoferum* + *Azotobacter chrococcoum*.

4. Dry weight of leaves (g)/plant:

Data illustrated in Table (4) reveal that, there were obvious effects of mineral nitrogen and bio-fertilizers on dry weight of leaves/plant of tuberose plants. The highest values recorded with the treatments of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, with highly significant differences compared with other treatments and control (100% mineral nitrogen) in both seasons. The lowest values in this regard were reported generally in case of 50% mineral nitrogen plus inoculation with *Asospirillum* in both seasons of study. Similarly, the effect of nitrogen fertilizer on dry weight of leaves of tuberose was studied by (Nain *et al*., 2018). While the positive response of herb dry weight by using nitrogen fixing bacteria was pointed out by Soliman *et al.* (2009) on French basil (*Ocimim bacilicum*, L.).

5. Fresh weight of root (g)/plant:

Data in Table (4) show that, the combined treatment of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, resulted in the highest and highly significant value over the other treatments and control (100% mineral nitrogen) with non-significant difference in the first season, and highly significant in the second one. These results agree with those obtained by Khalaj *et al.* (2012) on tuberos and Amin (1997) on coriander, fennel, and caraway.

6. Dry weight of root (g)/plant:

Data presented in Table (4) show that, there were highly significant effects due to the treatments, in most cases. Mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter,* gave the highest dry weight followed by 100% mineral nitrogen (control) during the two seasons, but with non-significant difference, between them in the first season. Furthermore, the differences between the combined treatment of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter* and other treatments were highly significant.

Florets characters:

1. Number of florets/spike:

According to data tabulated in Table (5) on number of florets/spike as affected by mineral nitrogen and bio-fertilization treatments, it could be concluded that, the highest effects were observed with the treatment of 100% mineral nitrogen (control), followed by mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, with non-significant difference between them in the 1st and 2nd seasons, respectively. The lowest values in this connection were reported generally in case of 50% mineral nitrogen plus inoculation with *Asospirillum* in both seasons.

Table 5. Effect of nitrogen fertilizer rates and bio-fertilizer kinds on number of floret and floret diameter on tuberose plants during the two seasons.

	Number of florets/plant		Floret diameter (cm)	
	1st season	2nd season	1st season	2nd season
50% N + S	19.00	20.67	2.93	3.02
50% N + Z	19.33	21.67	3.01	3.12
50% N + S*Z	20.67	22.67	3.14	3.27
75% N + S	24.33	24.33	3.22	3.46
75% N + Z	25.00	25.00	3.40	3.54
75% N + S*Z	26.33	27.67	3.54	3.63
100% N	26.67	28.00	3.32	3.49
LSD. at 5%	1.11	0.85	0.04	0.05
LSD. at 1%	1.56	1.20	0.05	0.07

N= nitrogen, S= *Azospirillum lipoferum*, Z= *Azotobacter chrococcoum* and S*Z= *Azospirillum lipoferum* + *Azotobacter chrococcoum*.

These results agree with those obtained by Khalaj *et al.* (2012) and Barman *et al* (2003) on tuberose.

2. Floret diameter:

The effect of mineral nitrogen fertilization and bio-fertilization kinds on floret diameter of tuberose plants was recorded in Table (5). The results reveal that, there were effects due to the treatments. Mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, gave the highest diameter with highly significant differences comparing with the other interaction treatments and 100% mineral nitrogen (control), during the two seasons. These results were in harmony with those reported by Patel (2005) on tuberose and Attia *et al.* (2005) on *Gladiolus grandiflorus*.

3. Fresh weight of spike (g)/plant:

Regarding the effect of nitrogen fertilizer rates and biofertilizer on the fresh weight of spike (g)/plant, the data illustrated in Table (6) reveal that, there were highly significant effects of the treatments on the fresh weight. The highest value was recorded with the treatment of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, comparing with the other treatments and control (100% mineral nitrogen), in both seasons. On the other hand, there was non-significant difference between mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter* and treatment with 100% mineral nitrogen (control) in the first season. The lowest values in this connection were reported generally in case of 50% mineral nitrogen plus inoculation with *Asospirillum* in both seasons These results were in parallel with those obtained by Patel (2005) on tuberose as affected by nitrogen fertilizer and Amin (1997) on coriander (*Coriandrum sativum*), fennel (*Feoniculum vulgare*), and caraway (*Carum carvi*) as influenced by bio-fertilizers.

4. Dry weight of spike (g)/plant:

Regarding tabulated data in Table (6) it may be noticed that, the combination treatments between different doses of mineral nitrogen fertilizers and bacteria strains affected dry weight of spike (g)/plant in both seasons. The heaviest and highly significant dry weight recorded with mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, treatment compared with the 100% mineral nitrogen (control) and other treatments, during the two seasons.

5. Length of spike (cm):

Data in Table (6) showed that, the interaction treatment of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, resulted in the tallest spike, with highly significant differences comparing with the other treatments and control (100% mineral nitrogen) treatment. These results were repeated in the second season, as the results of the first one.

Table 6. Effect of nitrogen fertilizer rates and bio-fertilizer kinds on number of floret, floret diameter, length of spike and longevity (days) of tuberose plants during the two seasons of 2017 and 2018.

	Fresh weight of spike/plant		Dry weight of spike/plant		Length of spike (cm)		Longevity (days)	
	1st season	1st season	1st season	1st season	1st season	2nd season	1st season	2nd season
50% N + S	89.7	101.0	22.5	22.4	13.33	16.00	13.53	14.07
50% N + Z	105.3	115.3	24.7	25.4	15.00	17.67	14.10	14.47
50% N + S*Z	127.7	132.0	29.4	30.2	18.67	19.00	14.73	14.77
75% N + S	146.3	150.0	32.9	33.9	18.33	19.67	14.97	14.93
75% N + Z	154.0	167.0	37.0	37.7	20.33	21.67	14.60	15.40
75% N + S*Z	183.0	199.7	43.4	44.3	22.33	23.67	15.77	16.67
100% N	179.3	194.3	40.3	42.9	20.67	21.67	14.70	14.93
LSD. at 5%	4.5	2.5	1.7	0.6	0.94	0.84	0.69	0.60
LSD. at 1%	6.3	3.6	2.4	0.9	1.32	1.18	0.97	0.84

N= nitrogen, S= *Azospirillum lipoferum*, Z= *Azotobacter chrococcoum* and S*Z= *Azospirillum lipoferum* + *Azotobacter chrococcoum*.

The lowest values in this connection were reported generally in case of 50% mineral nitrogen plus inoculation with *Asospirillum* in both seasons. These results agree with those reported by Khalaj *et al.* (2012) on tuberose and Attia *et al* (2005) on *Gladiolus grandiflorus*.

6. Longevity (days):

Data regarding the effect of mineral nitrogen levels and different kinds of bio-fertilization on longevity (days) were presented in Table (6). The treatments had pronounced influences on longevity. The differences between majority of treatments were highly significant. The superior effect obtained from the combined treatment of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, followed by mineral nitrogen at 75% plus inoculation with *Azotobacter* treatment, with highly significant effects during 2nd season. Furthermore, the treatment of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, recorded best results more than 100% mineral nitrogen (control) with highly significant differences during both seasons.

These results agree with those obtained by Kumar *et al.* (2017) and Ali *et al.* (2014) on gladiolus.

Chemical constituents:

1. Nitrogen percentage:

The effect of mineral nitrogen fertilization rates and bio-fertilization kinds on nitrogen percentage of tuberose leaves was recorded in Table (7). The results reveal that, there were clear effects due to different treatments. Mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter,* gave the highest and highly significant percentage of nitrogen compared to the other treatments, in both seasons. In the same way, the combined treatment of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, recorded an increase over the 100% mineral nitrogen (control) treatment with highly significant differences during the first and second seasons, respectively. These results were in harmony with those revealed by Almaliotis *et al.* (1997) who indicated that nitrogen content of upper and basal leaves of peach increased by increasing N level of nutrient solution and Gomaa and Abou-Aly (2001) on anise (*Pimpinella anisum*).

2. Phosphorus percentage:

Regarding the effect of mineral nitrogen fertilizer rates and bio-fertilization kinds on phosphorus percentage of tuberose leaves, the data illustrated in Table (7) reveal that, there were obvious effects in this regard. The highest values were recorded with the

Table 7. Effect of nitrogen fertilizer rates and bio-fertilizer kinds on number of N, P, K, carbohydrates percentage and chlorophyll a and b content in tuberose plants during the two seasons.

	N%		P%		K%		Chlorophyll A		Chlorophyll B	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
50% N + S	1.55	1.58	0.360	0.370	1.96	1.99	0.823	0.873	0.573	0.633
50% N + Z	1.63	1.68	0.393	0.410	2.06	2.15	0.850	0.907	0.617	0.673
50% N + S*Z	1.72	1.77	0.437	0.450	2.17	2.30	0.873	0.943	0.653	0.717
75% N + S	1.81	1.88	0.447	0.470	2.40	2.47	0.927	0.970	0.657	0.737
75% N + Z	1.84	1.92	0.463	0.487	2.57	2.65	0.953	1.013	0.757	0.767
75% N + S*Z	1.94	2.01	0.537	0.543	2.76	2.82	1.037	1.077	0.727	0.827
100% N	1.90	1.96	0.503	0.533	2.71	2.78	1.007	1.073	0.703	0.797
LSD. at 5%	0.016	0.017	0.007	0.009	0.03	0.03	0.007	0.018	0.078	0.017
LSD. at 1%	0.023	0.024	0.010	0.012	0.04	0.04	0.010	0.025	0.109	0.024

N= nitrogen, S= *Azospirillum lipoferum*, Z= *Azotobacter chrococcoum* and S*Z= *Azospirillum lipoferum* + *Azotobacter chrococcoum.*

treatments of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, with highly significant differences compared with other treatments in both seasons, and with 100% mineral nitrogen (control) treatment in the first season. The same trend was reported by Patel (2005) on tuberose and Gomaa and Abou-Aly (2001) on anise.

3. Potassium percentage:

Data presented in Table (8) show the effect of mineral nitrogen fertilizer rates and biofertilizers on potassium percentage. The treatment of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, resulted in the highest and highly significant value comparing with the other treatments and 100% mineral nitrogen (control) treatment during the two seasons, but the lowest values in this connection were reported generally in case of 50% mineral nitrogen plus inoculation with *Asospirillum* in both seasons of study. In harmony with these results were those revealed by Khalaj *et al.* (2012) on tuberos and Gomaa and Abou-Aly (2001) on anise.

4. Chlorophyll A percentage:

The effect of the treatments with mineral nitrogen fertilization rates and bio-fertilization kinds on chlorophyll A percentage of tuberose leaves was recorded in Table (7). The results reveal that, there were pronounced effects due to applying different treatments. The differences between combined treatments were highly significant. The highest percentage of chlorophyll A obtained from the treatment of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, furthermore, it gave the highest percentage over the control treatment with highly significant in the first season and non-significant in the second one. These results agree with those obtained by Khalaj *et al.* (2012) on tuberos and Attia *et al.* (2005) on *Gladiolus grandiflorus*.

5. Chlorophyll B percentage:

As for the treatments with mineral nitrogen fertilizer levels and different kinds of bio-fertilization on chlorophyll B percentage, the data registered in Table (7) show that, the treatment of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, resulted in the highest value with highly significant effect in both seasons, in most cases. On the same way, this treatment gave the highest percentage over the 100% mineral nitrogen (control) treatment with non-significant in the first season and highly significant in second one, respectively.

6. Carbohydrates percentage:

Data tabulated in Table (8), show that the highest effects of mineral nitrogen fertilization rates and different kinds of bio-

Table 8. Effect of nitrogen fertilizer rates and bio-fertilizer kinds on carbohydrates and essential oil percentages of tuberose plants during the two seasons.

	Carbohydrate %		Essential oil percentage	
	1st season	2nd season	1st season	2nd season
50% N + S	16.86	17.55	0.0250	0.0247
50% N + Z	17.09	17.94	0.0249	0.0251
50% N + S*Z	17.43	18.25	0.0252	0.0255
75% N + S	17.57	18.67	0.0254	0.0258
75% N + Z	17.92	18.92	0.0256	0.0258
75% N + S*Z	19.23	20.42	0.0268	0.0264
100% N	18.84	19.18	0.0261	0.0262
LSD. at 5%	0.14	0.23		
LSD. at 1%	0.19	0.32		

N= nitrogen, S= *Azospirillum lipoferum*, Z= *Azotobacter chrococcoum* and S*Z= *Azospirillum lipoferum* + *Azotobacter chrococcoum*.

fertilization on carbohydrates percentage in tuberose leaves were observed with the mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, followed by 100% mineral nitrogen (control) with highly significant difference between them in both seasons. These results agree with those reported by Khalaj *et al.* (2012) on tuberose and Attia *et al.* (2005) on *Gladiolus grandiflorus*.

7. Essential oil percentage:

According to data presented in Table (8) on essential oil percentage as affected by the treatments between mineral nitrogen fertilizer ratios and bio-fertilization kinds, it could be observed that, the highest result recorded with the treatment of mineral nitrogen at 75% plus the dual inoculation *Azospirillum* + *Azotobacter*, comparing with all treatments and followed by 100% mineral nitrogen (control) during the two seasons. This treatment yielded 0.0268 and 0.0264 %, in the first and second seasons, respectively. These results were in harmony with those revealed by Soliman *et al*. (2009) on volatile oil yield of french basil (*Ocimim bacilicum*, L.).

The correlation between longevity (day) and some flowering and vegetative characters:

The data in Table (9) show that, there were highly significant positive correlations between longevity (day) and all of floret diameter, length of spike, fresh and dry weights of spike, fresh and dry weights of leaves characters, under the treatments of mineral nitrogen fertilizer and bio-fertilizers, during the two seasons.

From the previous results we can indicate that, the increase in longevity (day) of the vase flowers, reflect positively the raise in floret diameter, spike length, fresh and dry weight of spike (g)/plant and fresh and dry weight of leaves (g)/plant under the treatments of mineral nitrogen fertilizer (50, 75 and 100% N) with bio fertilizers (*Azospirillum* and *Azotobacter*).

Recommendation:

From the aforementioned results, it could be recommended to treat tuberose plants (*Polianthes tuberosa* L.) grown in sandy soils with 75 % N + *Azospirillum lipoferum* + *Azotobacter chrococcoum* to obtain the highest parameters of vegetative growth characters, floral characters, the longevity of cut flower spikes (days) as well as the content of essential oil percentage in flowers.

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Table 9. Effect of N fertilizer and bio fertilizers treatments on the correlation measure between longevity (day) with some flowering and vegetative characters during the two seasons.

Flowering characters	Longevity (day)	
	1st season	2nd season
Floret diameter	0.963 **	0.966 **
Length of spike	0.999 **	0.999 **
Fresh weight of spike	0.962 **	0.969 **
Dry weight of spike	0.965 **	0.975 **
Fresh weight of leaves	0.949 **	0.997 **
Dry weight of leaves	0.959 **	0.939 **

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استجابة نباتات التيبروز للتسميد الحيوي والكيماوي وتأثيرهما علي النمو الخضري و الإزهار والتركيب الكيماوي تحت ظروف الأراضي الرملية

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أجريت تجربة حقلية بالمزرعة التجريبية لمحطة البحوث الزراعية بالقصاصين، مركز البحوث الزراعية، محافظة الإسماعيلية، مصر، لدراسة تأثير الاستبدال الجزئي للأسمدة النيتروجينية الكيميائية والإحلال بالتسميد الحيوي الذي يحتوي علي سلالتين من مثبتات النيتروجين (أزوسبيريلم ليبوفيرم ،أزوتوباكتر كروكوكم) على النمو الخضري والإزهار والتركيب الكيميائي لنباتات التيبروز تحت ظروف الاراضي الرملية لترشيد إستخدام الأسمدة المعدنية والحد من تلوث البيئة من أجل زيادة كمية وتحسين جودة المنتج من نباتات التيبروز خلال موسمين ناجحين فى عامي ٢٠١٧ و ٢٠١٨. إشتملت هذه التجربة سبع معاملات مكررة ثلاث مرات، وهي التوليفات بين معدلين من التسميد النيتروجين المعدني (٥٠٪ و ٧٥٪) وثلاثة من السماد الحيوي للبكتيريا المثبتة للنيتروجين (أزوسبيريلم ليبوفيرم، أزوتوباكتر كروكوكم و أزوسبيريلم + أزوتوباكتر) بالإضافة إلى معاملة المقارنة (١٠٠٪ نيتروجين). تم ترتيب المعاملات في تصميم قطاعات كاملة العشوائية. سجلت معاملة تسميد نباتات التيبروز بالنيتروجين المعدني بمعدل ١٠٠% من المعدل الموصي به أعلي القيم النباتية لارتفاع النبات، عدد الأوراق/نبات وعدد الزهيرات/شمراخ. بينما سجلت معامله التسميد بالنيتروجين المعدني بمعدل ٧٥% مع التلقيح بالازوسبيريلم + الازوتوباكتر أعلي القيم بالنسبة للوزن الطازج والجاف للأوراق، الوزن الطازج والجاف للجذر، الوزن الطازج والجاف للشمراخ الزهري، قطر الزهيرة، طول الشمراخ الزهري، طول عمر الأزهار (يوم) بالإضافة إلي محتوي الأوراق من النيتروجين، الفسفور، البوتاسيوم و كلوروفيل أ، ب وكذلك النسبة المئوية للكربوهيدرات الكلية وأيضا النسبة المئوية للزيت العطري وبدون فرق معنوي بينها وبين الكنترول في معظم الحالات في كلا الموسمين.