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Integrated Fertilizer Management Correctly Mirrors the Prospective Flower Quality, Bulb Yield and Beauty Features of Amaryllis (Hippeastrum Vittatum Herb)

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Abstract— *An innovative bioformulation labeled "Biofertile" of six endophytic diazotrophs was evaluated for improving flower quality, bulb yield and beauty features of amaryllis (Hippeastrum vittatum Herb). Along the winter growing seasons 2014-2015 and 2015-2016, leaf characteristics (numbers per plant, length, surface area, fresh and dry weights) significantly stimulated due to Biofertile treatment. Incorporation into soil of a rational N level with plant hormones did magnify the beneficial impact of the diazotroph formulation. Simultaneous bio-product inoculation and N fertilization resulted in earlier flower opening and budding, up to 18 days were resulted. The highest seasonal average bulb fresh weight of 76.9 g plant^{-1} was scored for plants inoculated with the diazotroph biopreparate together with 50 % N dose in presence of gibberellin and benzyl adenine. Addition of full N regime increased chlorophyll and carbohydrate contents of leaves by 26.8 and 16.7 % respectively over untreated amaryllis, Relatively low amounts of indole (0.56 mg/ 100 g fresh weight) and phenol (1.41 mg/ 100 g fresh weight) were estimated for untreated plants, higher quantities of 1.31 and 336 mg/ 100 g fresh weight were recorded for Biofertile-inoculated plants. Being among the beauty features of amaryllis, leaves area and fresh weight as well as bulb diameter and fresh weights conspicuously improved as the N content of the plant organs increased. Net N gains of 18.7 - 22.2 kg acre^{-1} were introduced into the ornamental plant growth media via inoculation with Biofertile either with or without the other additives. Results of this study open a new window on the successful role of the Integrated Fertilizer Management (IFM) concept for improving flower and bulb yields and characteristics with a unique contribution in the beauty features of amaryllis.*

Keywords—Amaryllis- Biofertile- N fertilizer- IFM- Flower quality- Bulb yield- Beauty features- N gain.

I. INTRODUCTION

Amaryllis (*Hippeastrum vittatum* Herb) belonging to the family Amaryllidaceae is a prominent ornamental and commercial flower of no actual rest-period if grown in warm weather as that of Egypt where it keeps its foliage evergreen all over the year. Besides its popularity as an ornamental garden plant with beautiful blossoms, flowers are used in perfume industry and also diuretic and emetic activities. Bulbs are used as well for curing raches in infants (Rammamurthy *et al*., 2010). The plant requires high nutrient supply for growth and development and to achieve the escalating target of good quality, the proper fertilization is utmost essential. Therefore, the integrated usage of the nutrients to get quality product without any environmental hazard is of prime concern. However, due to continuous and excessive use of inorganic fertilizers, the soil health is deteriorated. This also creates imbalance to environment by polluting air, water and soil. Application of organic manures as sources of nutrients with or without inorganic fertilizers seems to have great possibilities in avoiding or substituting the shear use of chemical fertilizers (Mazhabi *et al*., 2011). The use of organic amendments with microbial preparations along with judicious use of chemical fertilizers can improve biological and phyico-chemical properties of the soil, modifies nutrient uptake efficiency. Recently, microbial cultures proved to be an important component of integrated nutrient application in horticulture and seem a viable potential for efficient use of microbiota for maximizing crop production (Sajjad *et al*., 2014). Studies of Srivastava and Govil (2005) indicated the positive effect of

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inoculation with *Azotobacter* and phosphate solubilizing bacteria on vegetative and floral characteristics of gladiolus. Actually, the long season of amaryllis necessitates the adoption of an integrated nutrient system (INS) over a longer period for both vegetative and bulb growth. Since no reports are available on this research area for this special floral plant, the present study introduces original information on the complement between mineral fertilizer and an innovative diazotroph formation labeled "Biofertile" and to what extent this reflects on flower quality, bulb yield and beauty features of amaryllis.

II. MATERIALS AND METHODS

At the nursery of Ornamental Horticulture Department, Faculty of Agriculture, Cairo University; two pot experiments were executed during two successive seasons (2014/2015-2015/2016) of amaryllis cultivation.

Plant material

Amaryllis (*Hippeastrum vittatum*) mother bulbs obtained from Flomix, Giza, Egypt were selected to be similar in size (*ca.*33 mm diameter) and weight (*ca.*36 g) as possible and thoroughly washed in tap water. Bulbs were cultivated in the growth medium as one per pot.

Cultivation medium

A mixture of sand: silt: peat moss (1: 1: 1) was used as a growth medium.Plastic pots (25 cm diameter and 30 cm depth) were filled with the mixture at the rate of 3 kg pot^{-1}. Prior to planting, the potting medium was supplemented with PK fertilization regimes of 3 g pot^{-1} calcium superphosphate (P_2O_5, 15.5 %) and 1.5 g pot^{-1} potassium citrate ($C_6H_5K_3O_7$ (K_3O_7), 45 %), both are equivalent to the recommended application rates. Depending upon treatment, N in the form of ammonium sulphate ($(NH_4)_2SO_4$, 33.5 %) was incorporated into medium at either the recommendedlevel equivalent to 3 g pot^{-1} or its half. The NPK fertilizers were thoroughly mixed in the potting medium before planting.

Biofertilizer

A locally produced bacterial formulation innovated by the Environmental Studied and Research Unit (ESRU), Department of Microbiology, Faculty of Agriculture, Cairo University was the biofertilizer used. This bio-product, labeled as "Biofertile" is a composite culture of 6 potent associative diazotrophs (Table, 1). Those members are multifunctional and possess high N_2-fixation and plant hormone production capacities in addition to their ability to antagonize some plant pathogens.

For inoculation, uniform bulbs were soaked in freshly prepared Biofertile suspension for 30 min. to ensure sufficient coatingwith the diazotroph product, then left to air-dry in shade and planted in pots at *ca*. 3 cm depth. Extra 10 ml of the bioformulation was added over-head soil in the first watering. Boost inoculation took place twice at 3-week intervals of planting.

Table.1: Members of diazotrophs formulated in Biofertile

Strains	Code nos.	Host plants	References
Azospirillum brasilence	Azos. R7	*Ricinus communis*	Hamza *et al.* (1994)
Azotobacter chroococcum	Azot	*Hordeum vulgare*	Ali *et al*. (2005)
Bacillus polymyxa	B36	*Hamada elegans*	Hegazi and Fayez (2003)
Enterobacter agglomerans	K30	*Malva parviflora*	Hegazi and Fayez (2003)
Klebsiella pneumonia	MK9	*Zea mays*	Hamza *et al*. (1994)
Pseudomonas putida	Ps. G	*Sorghum biocolor*	Hamza *et al*. (1994)

Experimental layout

The different inoculation and mineral fertilization treatments were allocated in greenhouse in a complete randomized design with three replications. The applied design comprised, as well, the addition of the plant accelerators gibberellic acid (GA_3) and benzyl adenine (BA). The former was used as 200 or 100 mg l^{-1} and the latter at the concentration of 75 mg l^{-1}. The following six treatments were applied: 1) untreated bulbs, 2) recommended N fertilizer of 3 g pot^{-1} (RN), 3) RN +200 mg l^{-1} GA_3 +75 mg^{-1} BA, 4) Biofertile, 5) Biofertile+ ½ RN +200 mg l^{-1} GA_3 +75 mg^{-1} BA and 6) Biofertile + ½ RN +100 mg l^{-1} GA_3 +75 mg^{-1} BA. Along the experimental period extended to 6 months, pots were irrigated with a tap water when needed to maintain the soil water holding capacity at *ca*. 60 %. For the successive growth seasons 2014-2015 and 2015-2016, amaryllis planting date was 5th November for both.

Determinations

1- **Bacteriological analyses**

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The bacteriological determinations were limited for the enumeration of total bacterial and total diazotroph counts. Representative rhizosphere soil samples were collected after 30, 60, 90, 120and 180 days of planting and air-dried. A portion of 10 g soil was suspended in 90 ml sterile distilled water and serial decimal dilutions were prepared. Adopting the plate count technique, 1 ml aliquots from the proper dilutions were inoculated into nutrient agar (Atlas, 2010), or N-deficient combined carbon sources, CCM (Hegazi *et al*., 1998) media for total bacteria and total diazotrophs respectively. Plates were incubated at 30 °C for 2-4 days and CFUs were counted.

Acetylene reduction was assayed in amaryllis rhizosphere soils of the applied treatments at the end of the experiment using the procedure described by Fayez*et al.* (1983). Aliquots of 20 g air-dried soil unamended or amended with glucose (1 %) were placed in 120 ml flasks and watered up to *ca*.70 % WHC. Flasks were stoppered with serum caps pierced by needles and incubated in dark at 28 °C for 24 hr. Then, the needles were removed, 10 % air was replaced by acetylene, the flasks were reincubated for 2 hr. and acetylene reducing activity was measured by gas chromatograph.

2- **Vegetative growth parameters**

At flowering, leaves were determined for length, area as well as fresh and dry weights after drying at 70 °C to constant weight. And stems were estimated for length, diameter, fresh and dry weights. The flowering characteristics comprised the diameter, fresh and dry weights, time to bud and time to open.After flowers fading, plants were regularly watered until foliage began to turn yellow, then watering was stopped to let foliage die down. The soil was kept fairly dry until bulbs were dug out where the experiment was terminated to the measurements of bulb and root characteristics; bulb diameter, fresh and dry weights while roots for number as well as fresh and weights.

3- **Chemical constituents**

Chemical analyses of fresh and oven-dried leaves and bulbs included the photosynthetic pigments (chlorophyll and carotenoids) using the procedures described by Saric *et al*. (1967). Carbohydrates were assessed according to Herbert *et al*. (1971). Besides; N, P and K contents were determined according to Westerman (1990). Total phenols and indole acetic acid were estimated adopting the methods of Daniel and George (1972).

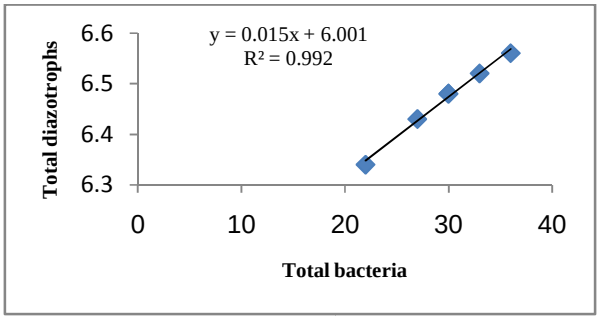
Statistical analysis

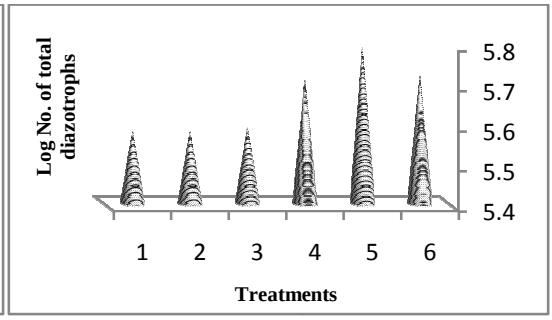
Data were subjected to statistical analysis of variance and means were compared using the least significant difference test at the 5 % level as described by Snedecor and Cocharan (1980). The linear regression and coefficient of determination among the main growth and yield parameters of the floral plant were estimated as well.

III. RESULTS

Effect of biological and chemical treatments on biological dynamics

Total bacterial and total diazotroph populations in the root theater of amaryllis proportionally increased with plant age up to 90 days, and slightly declined thereafter (data not shown). The average densities estimated along the growing seasons indicated that total bacteria were the highest for Biofertile-treatments (30-36 x10^5 cfu g^{-1}) particularly in presence of rational N fertilizer dose and plant hormones (Fig. 1). Untreated growth medium was rich enough to support high total bacterial accommodation, an average of 30 x10^5 cfu was recorded. Total diazotrophs successfully colonized the plant roots which hosted as high as > 6 x10^5 cfu g^{-1}. Amaryllis plants received Biofertile together with 50 % of recommended N level and plant hormones were the richest, those kept untreated were the poorest (< 40 x10^4 cfu g^{-1}). In general, this particular microbial community followed, among the applied treatments, an identical pattern to total bacterial population. This is indicated by the linear regression and coefficient of determination inserted in Fig. (1).





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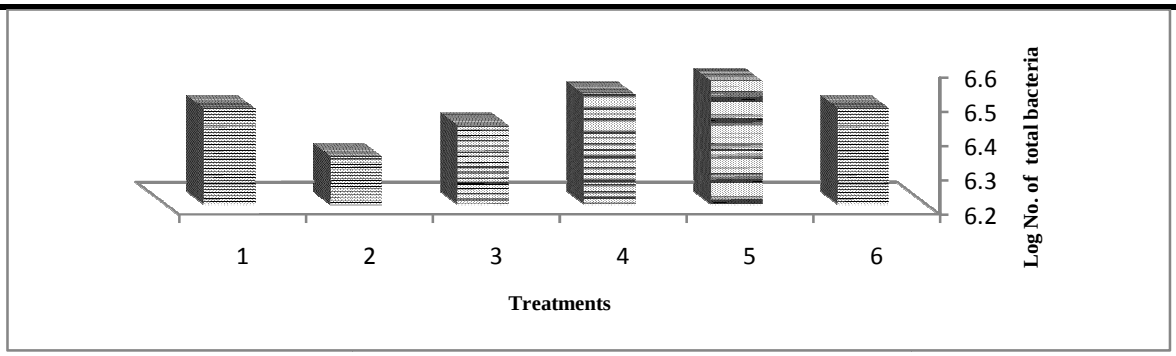

Fig. 1: Total bacterial and diazotroph populations in root theaterof thedifferent chemical and biological treatments. 1) untreated, 2) 3g N pot^{-1},3) 3g N pot^{-1} + 200 mgl^{-1} GA_3 +75 mgl^{-1} BA, 4) Biofertile, 5) Biofertile + 1.5 g N pot^{-1} + 200 mg l^{-1} GA_3 + 75 mg l^{-1} and 6) Biofertile + 1.5 g N pot^{-1} + 100 mgl^{-1} GA_3 +75 mgl^{-1} BA.

Negligible acetylene reducing activities (< 3 nmoles C_2H_4 g^{-1} h^{-1}) were measured for glucose-unamended growth medium. Storms of activities were scored in presence of the sugar. Autochthonous diazotrophs successfully fixed appreciable amounts of atmospheric dinitrogen that expressed in nitrogenase activity of 564.7 nmoles C_2H_4 g^{-1} h^{-1}, a record that significantly ($p \leq 0.05$) reduced to 99.2 when full N recommended regime was applied (Fig. 2). The bioformulation Biofertile was rich enough in N_2-fixing members to the extent of producing 786.3-933.3 nmoles C_2H_4 g^{-1} h^{-1}, the highest was in presence of the rational N dose, gibberellin and benzyl adenine.

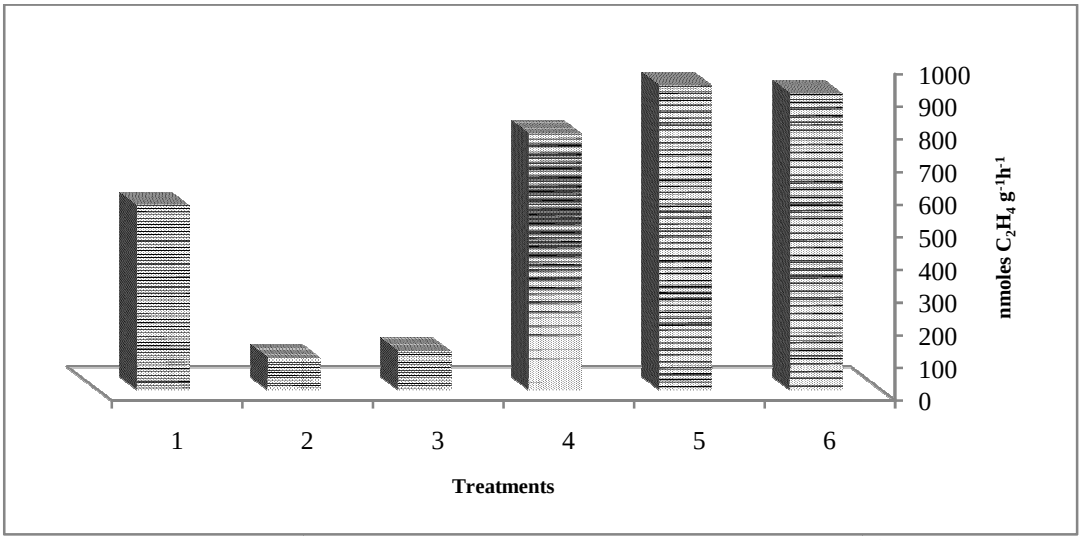

Fig. 2. Acetylene reducing activities in rhizosphere soils of amaryllis. 1) untreated, 2) 3g N pot^{-1},3) 3g N pot^{-1} + 200 mgl^{-1} GA_3 +75 mgl^{-1} BA, 4) Biofertile, 5) Biofertile + 1.5 g N pot^{-1} + 200 mg l^{-1} GA_3 + 75 mg l^{-1} and 6) Biofertile + 1.5 g N pot^{-1} + 100 mgl^{-1} GA_3 +75 mgl^{-1} BA.

Effect of treatments on vegetative traits

Based on the analysis of variance of amaryllis leaf characteristics, the effects of "Biofertile" either alone or simultaneously with mineral N fertilizer and plant hormones were statistically significant at 5 % level (Table, 2). This stimulatory influence extended for both growing seasons. In the majority of cases, gibberellin and benzyl adenine rather supported the beneficial impact of Biofertile.

Likewise, the inflorescence stalk parameters obviously promoted, an effect that was treatment-dependent (Table, 3). The diazotroph formulation did markedly overcome the full N application regime. In this context, stem biomass yield of the former ranged from 5.1 to 6.6 against 4.0 to 4.9 g plant^{-1} for the latter. Plant hormones concomitant to Biofertile were unavoidable to support amaryllis stem development.

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Table.2: Leaves characteristics of amaryllis as affected by biological and chemical treatments along the two successive seasons

Treatments	No. plant^{-1}	Length (cm)	Area (cm^2)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})
	2014-2015				
Control (nil N)	3.3	20.3	33.0	24.1	8.2
Control (RN)*	4.7	26.3	38.0	35.7	11.8
RN+GA_3**+BA***	4.7	38.0	53.9	39.5	12.7
Biofertile	5.0	50.0	98.7	44.3	15.3
Biofertile+1/2RN+ GA_3+BA	4.7	45.7	70.7	43.6	13.3
Biofertile+1/2RN+ 1/2GA_3+BA	5.0	52.3	98.2	48.0	16.9
LSD (0.05)	1.1	4.7	3.7	8.5	2.6
	2015-2016				
Control (nil N)	3.3	21.7	33.1	23.2	7.7
Control (RN)*	4.3	30.3	40.5	35.5	10.5
RN+GA_3**+BA***	4.7	41.7	60.5	42.5	12.5
Biofertile	4.3	54.7	97.0	50.1	16.5
Biofertile+1/2RN+ GA_3+BA	5.0	50.3	82.2	45.7	14.6
Biofertile+1/2RN+ 1/2GA_3+BA	4.7	55.5	114.8	51.9	16.9
LSD (0.05)	1.3	3.5	8.8	3.5	1.5

* RN, recommended N (ammonium sulphate, 3 g pot^{-1})

** GA_3, full gibberellin (200 mg l^{-1})

*** BA, benzyl adenine (75 mg l^{-1})

Table.3: Amaryllis inflorescence stalk properties due to the various biological and chemical treatments

Treatments	Length (cm)	Diameter (mm)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})
	2014-2015			
Control (nil N)	24.7	5.8	21.2	3.6
Control (RN)*	32.3	5.8	24.9	4.0
RN+GA_3**+BA***	34.7	6.3	27.7	4.9
Biofertile	40.3	9.4	32.1	5.2
Biofertile+1/2RN+ GA_3+BA	39.7	10.7	37.4	6.6
Biofertile+1/2RN+ 1/2GA_3+BA	41.0	8.5	31.7	5.1
LSD (0.05)	3.9	1.0	2.9	0.4
	2015-2016			
Control (nil N)	25.3	5.5	23.5	3.9
Control (RN)*	34.0	6.7	25.6	4.8
RN+GA_3**+BA***	31.7	6.6	27.2	4.7
Biofertile	38.0	9.0	34.8	5.5
Biofertile+1/2RN+ GA_3+BA	39.3	11.0	35.0	6.9
Biofertile+1/2RN+ 1/2GA_3+BA	37.0	8.9	33.4	5.1
LSD (0.05)	4.2	1.1	2.6	0.5

Effect of treatments on reproductive parameters

Data pertaining to flower properties revealed that Biofertile in combination with 50 % of the recommended N fertilizer rate together with gibberellin and benzyl adenine was the superior compared to others, a phenomenon noticed for both seasons

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(Table, 4). This was accompanied by earlier budding only in the first season, more than 20 days were required for bud formation during the second season. Fluctuations among the applied treatments in respect to flowers opening followed a trend akin to bud formation.

Table.4: Properties of amaryllis flowers as affected by biological and chemical treatments during the two seasons (changes related to 3 g N pot^{-1}-received plants)

Treatments	Diameter (mm)	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Time to bud (days)	Time to open (days)
	2014-2015				
Control (nil N)	-1.4	-3.1	-0.4	-17.0	-14.0
Control (RN)*	55.0	22.4	3.6	173.0-	179.0
RN+GA_3**+BA***	+0.6	-0.6	0.0	+4.0	+4.0
Biofertile	+17.5	+3.1	+0.6	-18.0	-15.0
Biofertile+1/2RN+ GA_3+BA	+38.9	+3.9	+1.1	-8.0	-5.0
Biofertile+1/2RN+ 1/2GA_3+BA	+33.7	+2.4	+0.7	-8.0	-7.0
LSD (0.05)	4.0	2.1	0.5	19.0	21.0
	2015-2016				
Control (nil N)	-8.0	-0.7	-0.2	-5.0	-6.0
Control (RN)*	55.8	22.2	3.6	146.0	153.0
RN+GA_3**+BA***	0.0	+1.0	+0.1	+7.0	+3.0
Biofertile	+20.0	+4.4	+0.6	+13.0	+15.0
Biofertile+1/2RN+ GA_3+BA	+39.4	+5.3	+1.1	+20.0	+22.0
Biofertile+1/2RN+ 1/2GA_3+BA	+33.6	+3.3	+0.9	+14.0	+13.0
LSD (0.05)	4.5	2.4	0.4	8.0	10.0

A perusal of data presented in Table (5) indicates that amaryllis root vegetative traits significantly responded to biological and chemical treatments. As high as 31 and 34 roots plant^{-1} were produced in the successive seasons due to Biofertile inoculation in presence of 50 % N and plant hormones. On the contrary, conspicuously lower numbers (16 and 20 roots plant^{-1}) were formed by 100 % N-received plants. Root fresh and dry weights similarly behaved among the different experimental treatments.

Table.5: Root parameters of amaryllis treated with Biofertile, ammonium sulphate and plant hormones during the two successive seasons

Treatments	No. plant $^{-1}$		Fw (g plant $^{-1}$)		Dw (g plant^{-1})	
	1st	2nd	1st	2nd	1st	2nd
Control (nil N)	12.0	14.0	10.5	11.4	3.5	3.8
Control (RN)*	16.3	19.7	12.1	12.0	4.0	4.2
RN+GA_3**+BA***	24.0	23.3	12.3	12.9	4.2	4.5
Biofertile	27.3	28.3	23.9	23.6	7.4	7.7
Biofertile+1/2RN+ GA_3+BA	30.7	33.7	26.9	27.0	8.4	8.6
Biofertile+1/2RN+ 1/2GA_3+BA	28.3	26.7	22.8	24.2	7.6	7.8
LSD (0.05)	2.2	2.9	1.2	1.9	0.3	0.5

Amaryllis bulb characteristics are the most prominent in relation to marketing and beauty. Results shown in Table (6) reveal that plants treated with the diazotroph bioproduct alone produced significantly bigger bulbs being 41.2 and 40.7 % higher than full N-supplied correspondings in 2014-2015 and 2015-2016 seasons respectively. Bulb diameters slightly improved due to simultaneous application of 50 % N and plant hormones. Biofertile activation on bulb development was rather high for both fresh and dry weights. The highest fresh weight of 50.7 g plant^{-1} (average of the two seasons) and dry weight of 15.1 g plant^{-1} were

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scored for plants received the bacterial preparation together with rational N level (50 %), gibberellin and benzyl adenine. As expected, the worst bulb properties were obtained for untreated amaryllis. Apart from growing season, Figure (3) illustrates the positive response of bulb to biological treatments compared to full N fertilization.

Table 6: Amaryllis bulb characteristics as affected by biopreparate application and chemical treatments (1st& 2nd seasons)

Treatments	Diameter (mm)		Fresh weight (g plant $^{-1}$)		Dry weight (g plant^{-1})	
	1st	2nd	1st	2nd	1st	2nd
Control (nil N)	29.2 (-26.3)	24.5 (-36.0)	21.5 (-25.1)	22.4 (-20.6)	6.1 (-24.7)	7.0 (-17.6)
Control (RN)*	39.6	38.3	26.9	28.2	8.1	8.5
RN+GA_3**+BA***	43.0 (+8.7)	45.8 (+19.6)	36.8 (+36.8)	41.5 (+47.2)	10.7 (+32.1)	12.0 (+41.2)
Biofertile	55.9 (+41.2)	53.9 (+40.7)	77.8 (+189.2)	73.5 (+160.6)	25.5 (+214.8)	25.1 (+195.3)
Biofertile+1/2RN+ GA_3+BA	51.8 (+30.8)	52.9 (+38.1)	51.7 (+92.2)	49.7 (+76.2)	15.4 (+90.1)	14.7 (+72.9)
Biofertile+1/2RN+ 1/2GA_3+BA	55.6 (+40.4)	56.2 (+46.7)	80.7 (+200.0)	73.2 (+159.6)	25.2 (+211.1)	26.0 (+205.9)
LSD (0.05)	3.9	4.5	7.3	4.9	1.8	1.0

-Values in parenthesis represent the change percentages related to recommended N-fertilized amaryllis.

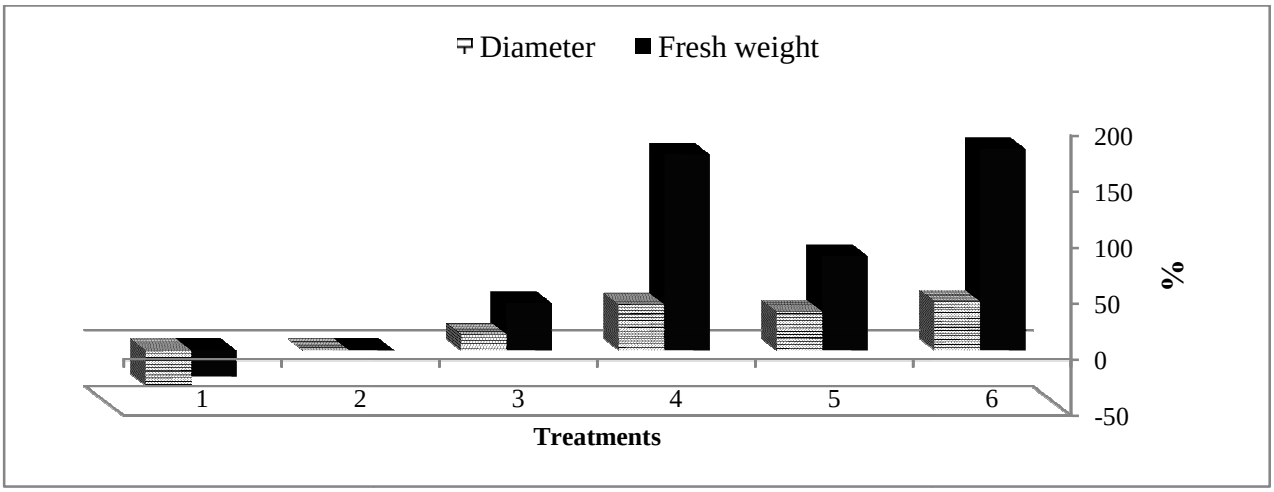

Fig. 3. Change percentages in bulb diameters and fresh weights due to biofertile and chemical treatments (related to 3 g N pot^{-1} - received plants). 1) untreated,2) 3g N pot^{-1},3) 3g N pot^{-1} + 200 mgl^{-1} GA_3 +75 mgl^{-1}BA,4) Biofertile,5) Biofertile + 1.5 g N pot^{-1} + 200 mg l^{-1} GA_3 + 75 mg l^{-1} and6) Biofertile+ 1.5 g N pot^{-1} + 100 mgl^{-1} GA_3 +75 mgl^{-1} BA.

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Effect of treatments on chemical attributes

Untreated plants depicted the lowest chlorophyll and carbohydrate contents in leaves (37.3 SPAD unit and 16.8 %, in average for both seasons)(Table,7). Incorporation into soil of full N dose resulted in increases of 26.3 and 14.9 % respectively. Significant increments in chlorophyll and carbohydrate pools were attributed to Biofertile whether in presence or absence of the other chemical additives.

The nutrient profile of leaves obviously improved due to the applied treatments particularly the biological ones (Table, 7). Low amounts of NPK were estimated for amaryllis left with no treatment, respective levels of 1.2, 0.22 and 1.6 % were recorded as averages of successive growing seasons. Higher quantities of the nutrients were accumulated in the plant leaves due to biological and chemical treatments, the former exceeded the latter in this respect. Biofertile alone or in conjugation with 50 %N and plant hormones deemed the pioneeric treatments in supporting the NPK pool of amaryllis leaves. The diazotroph product alone did successfully express itself as well with the accumulation of appreciable amounts of the nutrients in leaf tissues.

Table.7: Chemical profile of amaryllis leaves of the different experimental treatments during both seasons

Treatments	*Chlorophyll (SPAD unit)*	*Carbohydrates (%)*	*Nutrients (%)*		
			N	*P*	*K*
	2014 - 2015				
Control (nil N)	*36.5*	*17.1*	*1.2*	*0.23*	*1.2*
*Control (RN)**	*46.8*	*18.9*	*1.8*	*0.27*	*1.2*
*RN+GA_3**+BA****	*49.0*	*21.2*	*2.0*	*0.28*	*2.0*
Biofertile	*63.5*	*23.8*	*2.4*	*0.41*	*2.3*
Biofertile+1/2RN+ GA_3+BA	*67.3*	*21.6*	*2.0*	*0.35*	*2.4*
Biofertile+1/2RN+ 1/2GA_3+BA	*66.2*	*25.0*	*1.6*	*0.45*	*2.4*
LSD (0.05)	*4.5*	*1.3*	*0.2*	*0.1*	*0.3*
	2015 - 2016				
Control (nil N)	*38.1*	*16.4*	*1.2*	*0.21*	*1.9*
*Control (RN)**	*47.3*	*19.6*	*1.7*	*0.25*	*1.2*
*RN+GA_3**+BA****	*56.5*	*17.2*	*2.1*	*0.29*	*2.4*
Biofertile	*62.7*	*24.0*	*2.4*	*0.38*	*2.4*
Biofertile+1/2RN+ GA_3+BA	*66.6*	*22.5*	*1.9*	*0.41*	*2.4*
Biofertile+1/2RN+ 1/2GA_3+BA	*65.4*	*25.0*	*1.6*	*0.42*	*2.5*
LSD (0.05)	*4.4*	*1.5*	*0.3*	*0.11*	*0.2*

Significant increases in indole and phenol contents of leaves were attributed to all the applied treatments, biological treatments ranked the superior (Figure, 4). Mineral N fertilization alone enhanced indole and phenol production but to lower extents.

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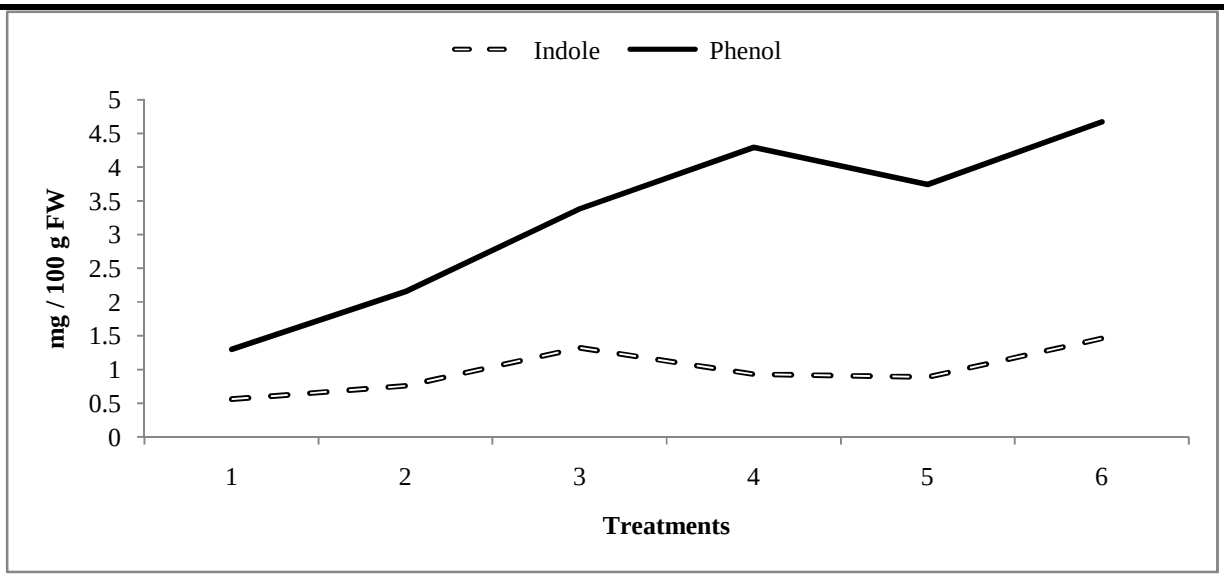

Fig. 4. Indole and phenol contents of amaryllis leaves of the different biological and chemical treatments. 1) untreated,2) 3g N pot^{-1},3) 3g N pot^{-1} + 200 mgl^{-1} GA_3 +75 mgl^{-1} BA,4) Biofertile,5) Biofertile + 1.5 g N pot^{-1} + 200 mg l^{-1} GA_3 + 75 mg l^{-1} and6) Biofertile + 1.5 g N pot^{-1} + 100 mgl^{-1} GA_3 +75 mgl^{-1} BA.

Similar to amaryllis leaves, carbohydrates and NPK levels in bulbs positively responded to the experimental treatments (Table, 8). Again, the bioformulation either alone or combined with chemical additives was the most stimulative for carbohydrate and nutrient accumulation in the plant bulbs.

Figure (5) illustrates that relatively low amounts of indole (0.56 mg /100 g fresh weight) and phenol (1.41 mg /100 g fresh weight) were estimated for bulbs of untreated plants, the quantities significantly raised as a result of 100 % N fertilizer application. Higher levels of 1.31 and 3.36 mg / 100 g fresh weight respectively were scored for Biofertile-inoculated amaryllis. Simultaneous addition of rational N regime and plant hormones resulted in the highest indole and phenol contents.

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Table.8: Carbohydrate and nutrient contents of amaryllis bulbs of the different treatments

Treatments	Carbohydrates (%)	Nutrients (%)		
		N	P	K
	2014-2015			
Control (nil N)	28.8	1.2	0.15	2.0
Control (RN)*	33.0	1.8	0.18	2.3
RN+GA_3**+BA***	31.9	2.0	0.21	2.8
Biofertile	36.7	2.4	0.30	2.8
Biofertile+1/2RN+ GA_3+BA	34.2	1.9	0.30	2.7
Biofertile+1/2RN+ 1/2GA_3+BA	38.6	1.6	0.34	2.8
LSD (0.05)	2.8	0.1	0.1	0.1
	2015-2016			
Control (nil N)	29.8	1.2	0.14	1.9
Control (RN)*	32.2	1.7	0.16	2.7
RN+GA_3**+BA***	33.4	2.1	0.24	2.8
Biofertile	36.8	2.5	0.32	2.8
Biofertile+1/2RN+ GA_3+BA	31.8	1.9	0.29	2.7
Biofertile+1/2RN+ 1/2GA_3+BA	37.4	1.5	0.31	2.9
LSD (0.05)	2.7	0.1	0.1	0.1

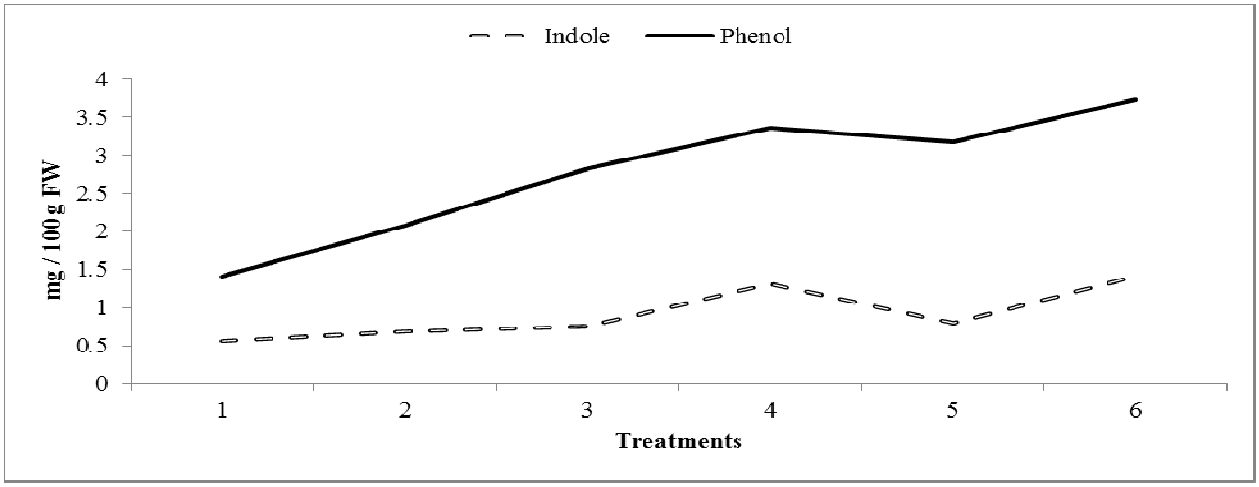

Fig. 5: Indole and phenol levels in amaryllis bulbs as affected by Biofertile, N fertilizer and hormone treatments.1) untreated,2) 3g N pot^{-1},3) 3g N pot^{-1} + 200 mgl^{-1} GA_3 +75 mgl^{-1} BA,4) Biofertile,5) Biofertile + 1.5 g N pot^{-1} + 200 mg l^{-1} GA_3 + 75 mg l^{-1} and6) Biofertile + 1.5 g N pot^{-1} + 100 mgl^{-1} GA_3 +75 mgl^{-1} BA.

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Table (9) summarizes the correlation matrix among some of the ornamental plant traits as affected by the applied treatments. The majority of assessed attributes were positively correlated to the significant levels (p< 0.05, 0.01) while the others were not.

Table.9: Correlation matrix (R values) among the major amaryllis growth parameters due tobiological and chemical treatments

	LA	SD	FD	FFW	RN	BD	BFW	BC	BN	BP	BK
LA	-	**0.78 ^{NS}**	**0.93****	**0.84***	**0.82***	**0.93****	**0.99****	**0.93****	**0.47 ^{NS}**	**0.98****	**0.79 ^{NS}**
SD		-	**0.93****	**0.98****	**0.97****	**0.98****	**0.74 ^{NS}**	**0.69 ^{NS}**	**0.61 ^{NS}**	**0.88***	**0.80 ^{NS}**
FD			-	**0.92****	**0.94****	**1.00****	**0.91***	**0.89***	**0.64 ^{NS}**	**0.96****	**0.93****
FFW				-	**0.95****	**0.92****	**0.81***	**0.71 ^{NS}**	**0.58 ^{NS}**	**0.92****	**0.74 ^{NS}**
RN					-	**0.93****	**0.77 ^{NS}**	**0.69 ^{NS}**	**0.60 ^{NS}**	**0.92****	**0.84***
BD						-	**0.91***	**0.89***	**0.99****	**0.96****	**0.93****
BFW							-	**0.94****	**0.88***	**0.94****	**0.78 ^{NS}**
BC								-	**0.51 ^{NS}**	**0.85***	**0.84***
BN									-	**0.51 ^{NS}**	**0.69 ^{NS}**
BP										-	**0.83***
BK											-

*Significant (p <0.05), **highly significant (p<0.01), ^{NS} non-significant.

LA, leaves area; SD, stem diameter; FD, flower diameter; FFW, flower fresh weight; RN, root number; BD, bulb diameter; BFW, bulb fresh weight; BC, bulb carbohydrates; BN, bulb nitrogen; BP, bulb phosphorus and BK, bulb potassium.

IV. DISCUSSION

Provided by good conditions and care, amaryllis plant will produce beautiful blooms year after year. The agrochemicals used excessively for its production did introduce major challenges for farmers in the form of soil infertility, nutrient imbalance, accumulation of toxic chemicals that have adverse effects on soil productivity, ecosystem destruction, environmental degradation which in turn affect the yield and quality of the final product. In that condition, sustainable agricultural practices have become a very difficult job for commercial growers nowadays.To cope with all these problems, cheaper and better technologies are necessary to improve soil fertility status, to maximize the floral plant productivity with minimum eco-hazards. All of these criteria can be achieved via the application of the Integrated Fertilizer Management “IFM” concept, that represents the use of biofertilizers together with rational mineral fertilizer regimes.

In the present study, a formulated bacterial product “Biofertile” entrapping 6 strains of associative diazotrophs (*Azospirillum brasilense*, *Azotobacter chroococcum*, *Bacillus polymyxa*, *Enterobacteragglomerans*, *Klebsiella pneumonia* and *Pseudomonas putida*) was experimented for amaryllis flower and bulb yield promotion. Data analysis indicated that leaf characteristics at maturity stage significantly improved due to inoculation with the diazotroph biopreparate, an effect that enhanced with simultaneous application of 50 % the field recommended level of N fertilizer.Full N regime, in absence of diazotroph product, supported as well the leaf properties but to lower extent. In conformity with these findings, Das *et al.* (2011) recorded significant increases in the tuberose (*Polianthes tuberose* Linn.) growth attributes; plant height, shoot number and leaf area due to biofertilizer application. Yadav *et al.* (2005) reported high tuberose plant height, shoot number and leaf area due to instant supply of NPK which improved synthesis and mobilization of metabolites to support faster vegetative growth. Studies of Koley and Pal (2011) proved significant increases in the tuberose leaf numbers and areas due to inoculation with *Azotobacter* or *Azospirillum* either alone or in combination. They attributed this effect to production of indole acetic acid, gibberellin, vitamin B12, thiamine and riboflavin (B2) by the used inocula. The superiority of leaf area among other vegetative traits is due to the fact that it is an indicator of photosynthesis, if leaf area is high, photosynthesis becomes

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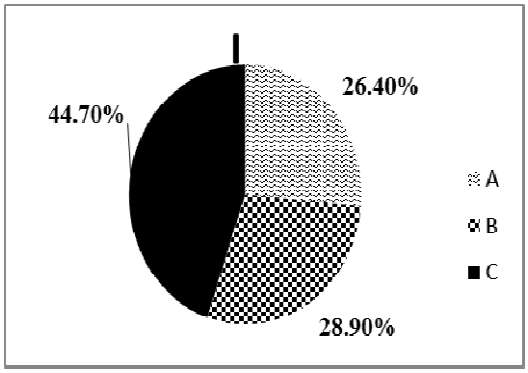
greater and metabolic materials are more available and consequently increases plant growth (Ahmadian *et al*., 2015). Indeed, the applications of plant growth promoting rhizobacteria (PGPR) particularly those fixing atmospheric dinitrogen include agriculture, horticulture, forestry and environmental restoration. The direct mechanism of plant growth booming by these bacteria encompasses nitrogen fixation for plant use, provision of bioavailable phosphorus for plant uptake, sequestration of iron for plants by siderophores, production of plant hormones such as auxins, cytokinins and gibberellins as well as lowering of plant ethylene levels. The indirect mechanisms are antibiotic protection against pathogenic bacteria, reduction of iron available to phytopathogenes in the rhizosphere, synthesis of fungal cell wall lysing enzymes and competition with detrimental microorganisms for sites on plant roots (Dilfuza, 2008). On the other hand, the beneficial effect of mineral N fertilization is attributed to the direct impact of the element, at a specific concentration, on new cell formation and thus increasing the number of leaves (Pal and Biswas, 2005). Furthermore, N might accelerate the decomposition of organic matter amendments, hence, increases might be occurred in water holding capacity and cation exchange capacity leading to deeper and more prolific plant root system besides better soil physical and chemical properties (Meshram*et al*., 2008). Additional reason for growth promotion due to Napplication is its role in forming important molecules including phospholipids, nucleotides, nucleic acids and certain co-enzymes which play a prominent role in plant metabolism and shortage of N results in the reduction of auxins necessary for plant growth (Mahmoodinezhadedezfully *et al*., 2012).

Data of the two seasons indicated that the heaviest amaryllis inflorescence stalk biomass yields resulted by Biofertile treatments particularly in presence of rational N level. Padaganur *et al*. (2010) explained increased biomass production due to N application as the element increases the availability of nutrients thereby stimulating development and size of photosynthesizingsurfaces and in turn more dry matter could be accumulated.

Flowering parameters positively responded to Biofertile inoculation with and/or without N and plant hormones. It is an interesting observation that biological and chemical treatments in combination forced the amaryllis flowers to bud and open up to 18 days earlier than the chemically-treated plants, a phenomenon that noticed only in the first growing season not the second. Similar results were obtained for gladiolus (Ali *et al*., 2014) where *Azospirillum* inoculation showed significant superiority taking 18 least days for sprouting. Gupta *et al*. (2004) reported that inoculation with VAM together with *Azospirillum* sp. and a phosphate solubilizing bacterium significantly reduced the number of days for first flowering. Also, EL-Mokadem and Sorour (2014) found that treatment of Petunia hybrid plants (cv. Bravo white) with full dose of NPK and ½ dose in combination with *Azospirillum lipoferum* plus *Bacillus polymyxa* inoculation forced the plants to flower profusely more than the other treatments due to improved vegetative growth which led to increased carbohydrates in plant tissues that are indispensable to initiate many flowering buds. Pirlak *et al*. (2007) explained the role played by biofertilizers in relation to flower initiation and duration as they lead to easy uptake of nutrients and simultaneous transport of growth promoting substances like cytokinines to the axillary buds resulting in breakage of apical dominance. Ultimately, they resulted in better sink for faster mobilization of photosynthates and early transformation of plant parts from vegetative to reproductive phase.

Amaryllis received the diazotroph formulation produced bigger and heavier bulbs, simultaneous application of 50 % of recommended N dose rather supported the bulb yields. These results confirm those reported for tuberose cv. Shringar where the biofertilizer of *Pseudomonas* sp. and *Trichoderma* sp. significantly increased bulb diameter, bulb weight and number of bullets (Srivastava *et al*., 2014).

Based on data mean comparison, Biofertile alone or with other chemical additives overcame the full N fertilization regime in respect to flower quality and bulb yield (Fig. 6). This observation opens new window to entrap potent microbial strains in the nutritional pools of the ornamental floral plants.



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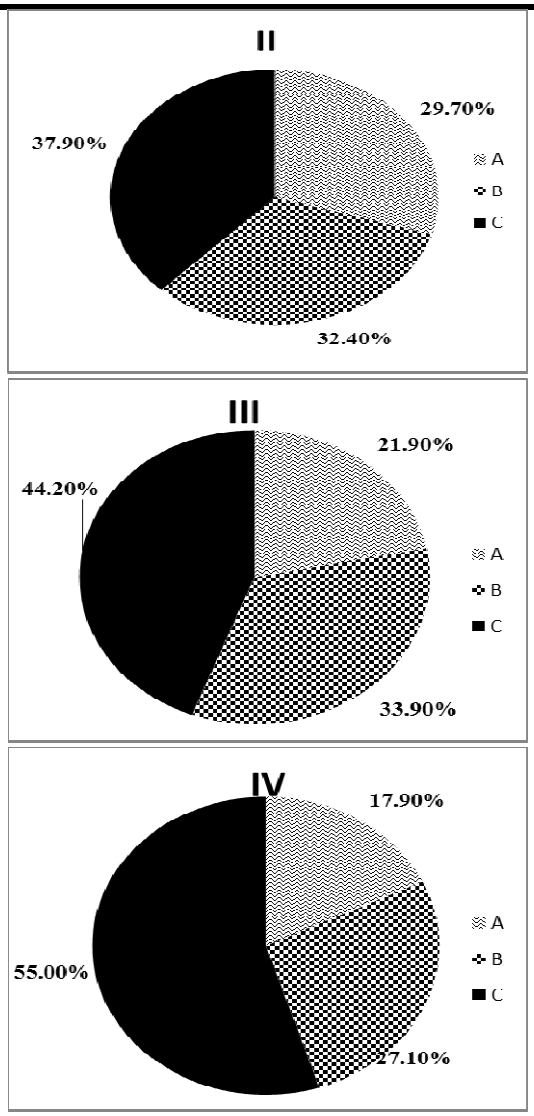

Fig. 6. Comparative flower and bulb quality of amaryllis plants among the different biological and chemical treatments. I, flower diameter; II, flower fresh weight; III, bulb diameter; IV, bulb fresh weight; A, untreated plants; B, chemically-treated plants; C, biologically-treated plants.

Chemical constituents of amaryllis obviously affected as well by Biofertile, N fertilizer and plant hormone treatments. In respect to chlorophyll and carbohydrate contents of leaves, increases of 33.5 and 22.4 % over full N-dressed plants were attributed to Biofertile alone. Simultaneous application of rational N level in presence of gibberellin and benzyl adenine supported higher accumulation of chlorophyll and carbohydrates in leaf tissues. Biofertilization of gladiolus with *Azotobacter*, *Azospirillum*, *Rhizobium* and phosphate solubilizing bacteria (Ali *et al*., 2014) resulted in more stored carbohydrates through effective photosynthesis. Actually, carbohydrates are the unique nutrients taking a major part in the development of flowers (Kumar and Haripriya, 2010). The NPK profile of the plant leaves and bulbs significantly increased due to all the applied treatments, those containing Biofertile ranked the pioneeric. In a number of cases, addition of gibberellin and benzyl adenine supported higher accumulation of the nutrients particularly in plant leaves. The higher quantities scored in biological treatments might be attributed to rapid absorption of these elements by the well-developed root system and their translocation to plant parts. Qasim *et al*. (2014)reported increases in NPK of gladiolus leaves and bulbs as a result of inoculation with *Azospirillum*, *Azotobacter*, *Rhizobium* and phosphate solubilizing bacteria.

Biofertile and N fertilization conspicuously stimulated indole acetic acid and phenols production in amaryllis leaves and bulbs. These substances modulate several growth and developmental process viz., cell division, differentiation, flowering fruit ripening, emberyogensis, senescence and rhizogensis (Kakkar *et al*., 2000).

Leaves and bulbs are among the attracting organs of the ornamental plant, so it was of rather interest to assess the relationship between some of their beauty features with the corresponding N pool, a unique parameter that correctly mirrors the real impact of N fertilization and diazotroph inoculation. Figures (7&8) illustrate the significant positive correlations between the N pools of leaves and bulbs with a number of their beauty features.

Regarding the C_2H_2 reduced /N_2 fixed conversion factor of 3 assumed by Hardy *et al.* (1968), the highest estimate of acetylene reducing activity of 933.3 nmoles C_2H_4 g^{-1} h^{-1} reported for amaryllis treated with Biofertile in combination with half N level, gibberellin and benzyl adenine represents a gain of 22.2 kg N $acre^{-1}$.Appreciable amounts of N (18.7 kg $acre^{-1}$) were added to soil N pool *via* Biofertile treatment alone. In absence of diazotroph formulation and plant hormones, full N fertilization regime diminished the net soil N gain to 2.4 kg $acre^{-1}$. These findings introduce a clue on the necessity of diazotroph inoculation not only to improve the yield and beauty features of the ornamental plant but to furnish the soil N budget with extra quantities of the element as well.

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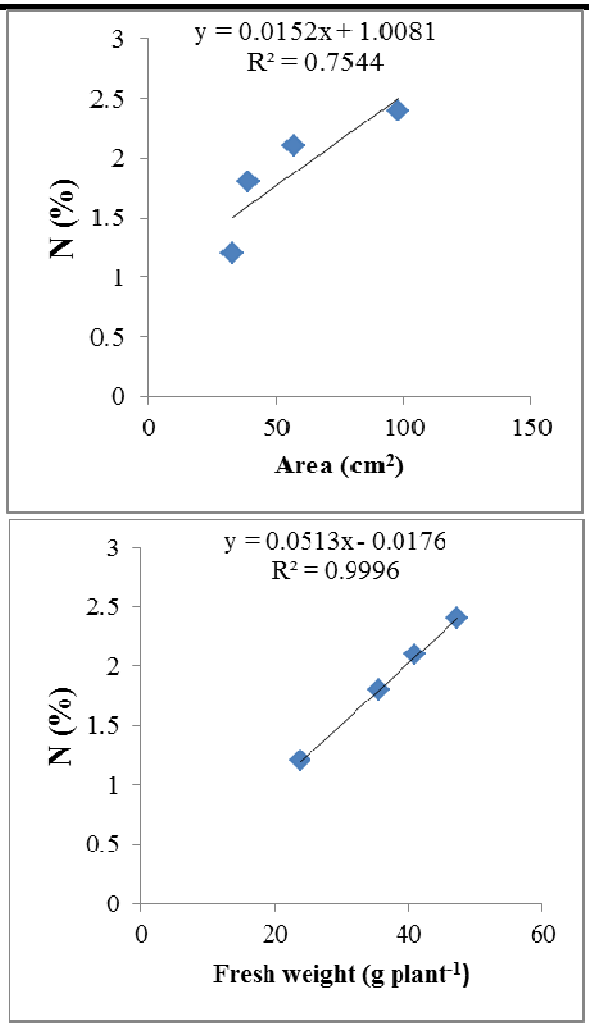
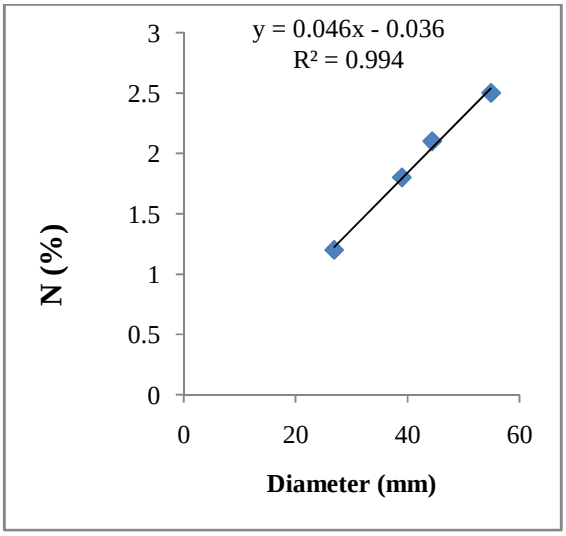


Fig. 7: Linear regression and coefficient of determination among amaryllis leavesN content with either area or fresh weight.



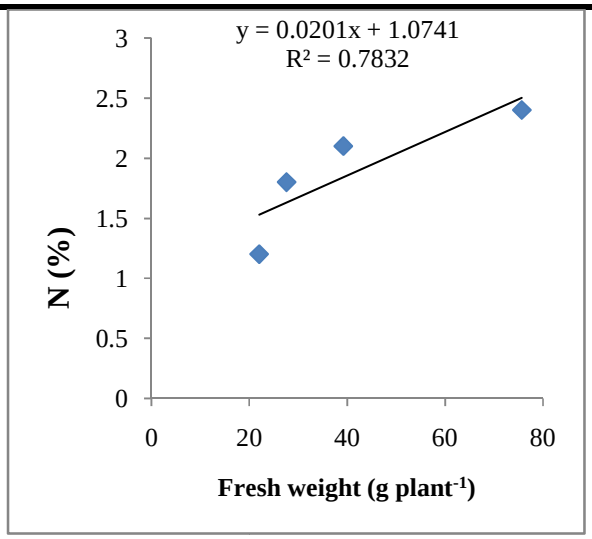


Fig. 8: Relationships between amaryllis bulb N content and either diameter or fresh weight

In conclusion, it should be realized that, understanding the mechanisms of ornamental plant growth promotion is of special concern when deciding what types of bacteria should be used with a plant in a given situation. What is currently missing from research on diazotroph formulations in horticulture is the lack of comparative studies between crop types and different species or strains of rhizobacteria. What is needed for the future in this area of research is a clear definition of what bacterial traits are useful and necessary for various environmental conditions and plants, so that optimal bacterial candidates could either be selected or constructed. In addition, it would be unavoidable to have better understanding of how different bacterial strains work together, in a composite, for the synthetic promotion of plant development.

REFERENCES

[1] Ali, A.; Mehmood, T.; Hussain, R.; Bashir, A.; Raza,S.; Din, N. and Ahmad, A. (2014).Investigation of biofertilizers influence on vegetative growth, flower quality, bulb yield and nutrient uptake in gladiolus (*Gladiolusgrandiflorus* L.). Inter. J. Plant, Animal, Environ. Sci., 4: 94-99.

[2] Ali, M.S.; Hamza, A.M.; Amin, G.; Fayez, M.; EL-Tahan, M.; Monib, M. and Hegazi, N.A. (2005). Production of biofertilizers using baker's yeast effluent and their application to wheat and barley grown in north Sinai deserts. Arch. Agron. Soil Sci., 51: 589-604.

[3] Ahmadian, Leila; Arab, M. and Roozban, M. R. (2015).Impact of biological fertilizers on vegetative

https://dx.doi.org/10.22161/ijaers/3.10.19 *ISSN: 2349-6495(P) | 2456-1908(O)*

and reproductive traits of stock (*Mathiola incana* L.) Inter. J. Agric. Crop Sci., 8(3):477-481.

[4] Atlas, R.M. (2010).Handbook of Microbiological Media. Fourth edition. CRC Press, Inc., 6000 Broken Sound Parkway NW, Suite 300,Boca Raton, Florida, 33487-2742.

[5] Daniel, H.D and George, C.M. (1972).Peach seed dormancy in relation to indigenous inhibition and applied growth substances. J. Amer.Soc. Hort. Sci., 97:651-654.

[6] Das, P.; Paswan, L.; Choudhury, H.; Das, J. and Saikia, P. (2011).Effect of inorganic, organic and biofertilizers on yield and yield attributes of tuberose (*Polianthes tuberose* Linn.). India Crop Res., 42 (1, 2 & 3): 227-230.

[7] Dilfuza, E., 2008.Plant growth promoting properties of rhizobacterial isolates from wheat and pea grown in loamy sand soil. Turk. J. Biol., 32: 9-15.

[8] El-Mokadem, E. Hoda and Sorour, Mona (2014).Effect of bio and chemical fertilizers on growth and flowering of *Petunia hybrida* plants. American, J. Plant Physiol., 9(2):68-77.

[9] Fayez, M.; Emam, N.F. and Makboul, H.E. (1983).Interaction of the herbicides Bromoxynil and Afalon S with *Azospirillum* and growth of maize, Z. Pflanzenernahr. Bodenk., 146:741-751.

[10] Gupta, Y.C.; Suman, B.; Sharma, Y.D.; Thakue, R. and Ritu, J. (2004).Effect of growing media and fertilization on growth and flowering of carnation (*Dianthus caryophyllus* L.) under protected conditions. Proceedings of the National Symposium on Recent Trends and Future Strategies in Ornemental Horticulure, Dharwad, India, December 1-4, p.77.

[11] Hamza, M.A.; Youssef, H.; Helmy, A.; Amin, G.A.; Fayez, M.; Higazy, A.; El-Khawas, H.M.; Monib, M.; Sedik, M.Z. and Hegazi, N.A. (1994). Mixed cultivation and inoculation of various genera of associative diazotrophs. In: Hegazi, N. A; Fayez, M. and Monib, M. (eds.), Nitrogen Fixation with Non-Legumes. The American University in Cairo Press, Cairo, Egypt, pp: 319-326.

[12] Hardy, R.W.F.; Holsten, R.D.; Jackson, E.K. and Burns, R.C. (1968).The acetylene-ethylene assay for N_2-fixation: Laboratory and field evaluation, Pl. Physiol., 43:1185-1207.

[13] Hegazi, N.A. and Fayez, M. (2003). Biodiversity and endophytic nature of diazotrophs other than rhizobia associated to non-leguminous plants of semi-arid environments. Arch. Agron. Soil Sci., 49: 213-235.

[14] Hegazi, N.A.; Hamza, A.M.; Osman, A.; Ali, S.; Sedik, M.Z. and Fayez, M. (1998). Modified combined carbon N-deficient medium for isolation, enumeration and biomass production of diazotrophs. In: Nitrogen Fixation with Non-Legumes. (Malik, K.A., Mirza, M.S. and Ladha, J.K., eds). Kluwer Academic Publishers, pp: 247-253.

[15] Herbert, D.P.; Phlipps, P.and Strangl, R. (1971).Determination of Carbohydrates.Meth.Microbial., 58: 209-344.

[16] Kakkar, R.K.; Nagar, P.K.; Ahuja, P.S. and Rai, V.K. (2000). Polyamines and plant morphogenesis. Boil. Plant, 43: 1-110.

[17] Koley, S. and Pal, A. K. (2011).Response of inorganic fertilizer & biofertilizer on growth and flower yield of tuberose (*Polianthes tuberosa* L.) cv. Prajwal in the plains of West Bengal. J. Crop and Weed 7(2):241-243.

[18] Kumar, S. and Haripriya, K. (2010).Effect of growth retardants on growth, flowering and yield of Nerium *(Nerium odorum* L.*).* Plant Arc., 10: 681-684.

[19] Mahmoodinezhadedezfully, S.H.; Gholami, A.; Moezi, A.A. and Hosseinpour, M. (2012).Effects of nitrogen, potassium and phosphorus on quantitative and qualitative characteristics of Tuberose Cv. Double (*Polianthes Tuberosa L*) J. Appl. Environ. Biol. Sci., 2(9) 485-491.

[20] Mazhabi, M.; Nemati, H.; Rouhani, H.; Tehranifar, A.;Mahdikhani, E. andKaveh, H. (2011). How may *Trichoderma* application affect vegetative and qualitative traits in Tulip "Darwin Hybride" cultivar. J. Biol. Environ. Sci., 5(15), 177-182.

[21] Meshram, N.; Badge, S.; Bhongle, S.A. and Khiratakar, S.D.(2008).Effect of bioinoculants with graded doses of NPK on flowering, yield attributes and economics of annual chrysanthemum. J. Soils Crops, 18: 217-220.

[22] Padaganur, V.G.; Mokashi, A.N. and Patil, V.S. (2010).Flowering, flower quality and yield of tuberose (*Polianthes tuberosa* L.) as influenced by vermicompost, farmyard manure and fertilizers. Karnataka, J. Agric. Sci., 18: 729-734.

[23] Pal, A.K. and Biswas, B. (2005). Response of fertilizer on growth and yield of tuberose (*Polianthes tuberosa* L.) cv. Calacutta single in the plains of Wast Bengle. J. Interacademicia, Nadia, India, 9(1): 33-36.

[24] Pirlak. L.; Turan, M; Shhin, F. and Esitken, A. (2007).Floral and foliar application of plant growth promoting rhizobacteria (PGPR) to apples increases

https://dx.doi.org/10.22161/ijaers/3.10.19 *ISSN: 2349-6495(P) | 2456-1908(O)*

yield, growth and nutrition element contents of leaves. J. Sustain. Agric., 30: 145-156.

[25] Qasim, M.; Younis, A.; Zahir, Z. A.; Riaz, A.; Raza, H. and Tariq, U. (2014).Microbial inoculation increases the nutrient uptake efficiency for quality production of *Gladiolus grandiflorus*. Pak. J. Agric.Sci.,51(4): 875-880.

[26] Rammamurthy, S.; Venkataraman, S.; Meera, R.; Satkar, P.; Chriristina, A.J.M. and Devi, P. (2010). Phytochemical investigation of *Polianthes tuberosa*.Inter. J. Pharm. Tech. Res., 2 (2): 1204-1206.

[27] Sajjad, Y.; Jaskani, M.J.; Ashraf, M.Y.; Qasim, M. and Ahmad, R. (2014).Response of morphological and physiological growth attributes to foliar application of plant growth regulators in gladiolus "White Prosperity". Pak. J. Agri. Sci., 51:123-129.

[28] Saric, M.R., Kostrori, T. Gupina and Geris, I. (1967). Chlorophyll determination Univ. Sadu Prakitikum is kiziologize Bilijaka- Beagrad,Haucua Anjiga, pp. 215.

[29] Snedecor, G. and Cochran, W. (1967)."Statistical Analysis" 6 ed. Iowa State Univ. Press, Ames, Iowa.,USA.

[30] Srivastava, R. and Govil, M. (2005). Influence of biofertilizers on growth and flowering in gladiolus cv. American Beauty. Acta Hort. 742:183-188.

[31] Srivastava, R.; Preetham, S.P. and Chand, S. (2014).Effect of organic manures and biofertilizers on vegetative, floral and post-harvest attributes in Tuberose (*Polianthes tuberosa*) var. Shringar. Asia. J. Biol. Life, Sci., 6-9.

[32] Yadav, B. S.; Gupta, A. K. and Singh, S. (2005).Studies on the effect of nitrogen, plant spacing and biofertilizers on parameters in Tuberose cv. Double. Haryana J. Horti.Sci., 34: 78-80.

[33] Westerman, R.L. (1990). Soil Testing and Plant Analysis. (3rd ed) Soil Science Society of America, Inc. Madison.