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Effect of biostimulants and biofertilizers on growth, flowering and quality of gladiolus (*Gladiolus grandiflorus* L.) Cv. American beauty under greenhouse conditions

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Abstract
The present experiment entitled "Effect of biostimulants and biofertilizers on growth, flowering and quality of gladiolus (*Gladiolus grandiflorus* L.) cv. American Beauty under greenhouse conditions" was carried out at High Tech Horticulture Park, Department of Horticulture, College of Agriculture, Junagadh Agricultural University, Junagadh during 2015-2017. The experiment was laid out in Factorial Completely Randomized Block Design (FCRD) consisting two factors with three repitations. Four treatments of biostimulants and three treatments of biofertilizers were considered as treatment combinations. The results indicated that application of humic acid 0.2% with *Azotobacter* @ 2.5 ml/plant + PSB @ 2.5 ml/plant + KSB @ 2.5 ml/plant at first spray at 30 DAP, second spray at 45 DAP and third spray at 60 DAP of biostimulants and soil application of biofertilizers at the time of planting and two month after planting gives higher vegetative growth, flowering and quality parameters in gladiolus.

Keywords: Gladiolus, corms, cormels, biostimulants, biofertilizers

Introduction
Gladiolus (from Latin, the diminutive of gladius, a sword) is a genus of perennial bulbous flowering plants in the iris family (Iridaceae). Sometimes called the sword lily, the most widely used English common name for these plants is simply gladiolus (plural gladioli, gladioluses or sometimes gladiolas). Gladiolus could be grown in any type of soil provided it is well drained. For good performance, it prefers a sandy loam soil, rich in organic matter. A soil pH between 6 and 7 is ideal; however, a soil with pH ranging from 5 to 7 can also be used for gladiolus cultivation. In soil by adding organic manures and microbial agents make easy uptake of nutrients when crop required comparing to chemical fertilizers (Vanilarasu and Balakrishnamurthy, 2014). Mineral nutrition does play an important role in influencing the quality of crops and it is fact that the soil health deteriorates due to continuous use of chemical fertilizers. The use of biostimulants and biofertilizers improves physico-chemical and biological properties of soil, besides improving the efficiency of applied nutrients. In case of biostimulants, Salukhe (2010) [10] analysed the samples of banana pseudostem for its elemental composition and found that banana pseudostem contained macro elements in the range of 1.00 to 1.12 % N, 0.50 to 0.71 % P, 2.39 to 2.62 % K and micro nutrients in the range of 259 to 323.2 mg/kg Fe, 47.3 to 241.3 mg/kg Mn, 10.1 to 107.4 mg/kg Zn and 13.4 to 83.6 mg/kg Cu. Seaweed extracts act as biostimulants mainly due to the presence of plant hormones. Main phytohormones identified in seaweed extracts are: auxins, cytokinins, gibberelins, abscisic acid and ethylene. Panchagavya consists of nine products viz. cow dung, cow urine, cow milk, curd, jaggery, ghee, banana, tender coconut and water. When suitably mixed and used, these have miraculous effects. Physico-chemical properties of panchagavya revealed that they possess almost all the major nutrients, micro nutrients and growth harmones (IAA & GA) required for crop growth. It contain properties like naturally occurring, beneficial, effective microorganisms, predominately lactic acid bacteria, yeast, actinomycetes and photosynthetic bacteria. Humic acid that promote plant health and growth. The importance of humic acid lies in their ability to promote hormonal activity in plants. Plant hormones are chemical communicators, or agents, which help regulate a plant's development and its response to its surrounding environment. Humic acids also promote antioxidant production in plants which, in turn, reduces "free radicals".

Free radical molecules result from stress such as drought, heat, ultraviolet light and herbicide use. Free radicals are damaging because they are strong oxidizing agents which damage lipids, proteins and DNA within plants cells. Antioxidants are metabolites and enzymes which seek out free radical molecules and protect plants from damage. They include lipid soluble substances like vitamin "E" and beta-carotene and water soluble materials such as vitamin C and various enzymes. In case of biofertilizers, which have a ability to enrich the soil with beneficial microorganisms as well as to mobilize the nutritionally important elements from non-usable to usable forms through biological processes resulting in enhanced production of flowers, fruits and vegetables offer an alternative (Purkayastha *et al*., 1998) [19]. The use of biofertilizers in combination with chemical fertilizers and organic manures offers a great opportunity to increase the production as well as quality of gladiolus. Among the nitrogen fixing bacteria, *Azotobacter*, not only provides nitrogen, but also synthesizes growth promoting hormones such as IAA and GA. *Azospirillum* also helps in plant growth and increases the yield of crops by improving root development, mineral uptake etc. The positive role of these biofertilizers has been recorded in many vegetables and spice crops by different scientists. To maintain long term soil health and productivity there is a need for integrated nutrient management through manures and biofertilizers apart from costly chemical fertilizers for better yield of the crop (Mondel *et al*., 2003) [15]. considering the above facts, the present study was planned and undertaken with the objective to assess the effect of biostimulants and biofertilizers on growth, flowering and quality of gladiolus.

Materials and Methods

The field experiment entitled "Effect of biostimulants and biofertilizers on growth, flowering and quality of gladiolus (*Gladiolus grandiflorus* L.) cv. American Beauty under greenhouse conditions" was carried out at High Tech Horticulture Park, Department of Horticulture, College of Agriculture, Junagadh Agricultural University, Junagadh during 2015-2017. The trial was laid out in Factorial Completely Randomized Block Design (FCRD) with 12 treatments and three replication. The different treatments were T_1: Banana pseudo stem sap 1% + *Azotobacter* @ 1.5 ml/plant + PSB @ 1.5 ml/plant + KSB @ 1.5 ml/plant, T_2: Banana pseudo stem sap 1% + *Azotobacter* @ 2 ml/plant + PSB @ 2 ml/plant + KSB @ 2 ml/plant, T_3: Banana pseudo stem sap 1% + *Azotobacter* @ 2.5 ml/plant + PSB @ 2.5 ml/plant + KSB @ 2.5 ml/plant, T_4: Seaweed extract 1% + *Azotobacter* @ 1.5 ml/plant + PSB @ 1.5 ml/plant + KSB @ 1.5 ml/plant, T_5: Seaweed extract 1% + *Azotobacter* @ 2 ml/plant + PSB @ 2 ml/plant + KSB @ 2 ml/plant, T_6: Seaweed extract 1% + *Azotobacter* @ 2.5 ml/plant + PSB @ 2.5 ml/plant + KSB @ 2.5 ml/plant, T_7: Panchgavya 3% + *Azotobacter* @ 1.5 ml/plant + PSB @ 1.5 ml/plant + KSB @ 1.5 ml/plant, T_8: Panchgavya 3% + *Azotobacter* @ 2 ml/plant + PSB @ 2 ml/plant + KSB @ 2 ml/plant, T_9: Panchgavya 3% + *Azotobacter* @ 2.5 ml/plant + PSB @ 2.5 ml/plant + KSB @ 2.5 ml/plant, T_{10}: Humic acid 0.2% + *Azotobacter* @ 1.5 ml/plant + PSB @ 1.5 ml/plant + KSB @ 1.5 ml/plant, T_{11}: Humic acid 0.2% + *Azotobacter* @ 2 ml/plant + PSB @ 2 ml/plant + KSB @ 2 ml/plant, T_{12}: Humic acid 0.2% + *Azotobacter* @ 2.5 ml/plant + PSB @ 2.5 ml/plant + KSB @ 2.5 ml/plant.

Time of application of biostimulants (spray)	Time of application of biofertilizers (Drenching)
1. 1st spray at 30 DAP	1. 1st - At the time of planting
2. 2nd spray at 45 DAP	2. 2nd - 2 month after planting
3. 3rd spray at 60 DAP	

Result and Discussion

Table 1: Effect of biostimulants

The data revealed that the significantly maximum plant height at 90 DAP (113.01 cm), number of leaves per plant (7.66), number of tillers per plant (2.06), fresh weight of plant (131.38 g), dry weight of plant (38.28 g) and minimum days taken for opening of first spike (55.41) were registered with an application of humic acid 0.2% (B_4) in pooled. Plant growth is also improved by the ability of the plant to uptake and receive more nutrients. Humic acid is especially beneficial in freeing up nutrients in the soil so that they are made available to the plant as needed. For instance if an aluminum molecule is binded with a phosphorus one, humic acid detaches them making the phosphorus available for the plant. Humic acid is also especially important because of its ability to chelate micronutrients increasing their bio-availability. Humic acid stimulates microbial activity by providing the indigenous microbes with a carbon source for food, thus encouraging plant's growth and activity. Soil microbes are responsible for solubilizing vital nutrients such as phosphorus that can then be absorbed by the humic acid and in turn made available to the plant for better growth and root development. Increased growth of plant in present study are in agreement with the results obtained by Sankari *et al*. (2015) in gladiolus; Khenizy *et al*. (2013) in gerbera; Yasser *et al*. (2011) in hibiscus and Vijayalakshmi and Mathan (1997) in sunflower.

Effect of Biofertilizers

The data revealed that the maximum plant height at 90 DAP (110.89 cm), number of leaves per plant (7.58), number of tiller per plant (2.06), fresh weight of plant (127.91 g), dry weight of plant (36.43 g) and minimum days taken for opening of first spike (55.94) were recorded with an application of *Azotobacter* @ 2.5 ml/plant + PSB @ 2.5 ml/plant + KSB @ 2.5 ml/plant (F_3) in pooled. This may be due to application of biofertilizer. *Azotobacter* is an aerobic free living bacterium and has specific role in fixing atmospheric nitrogen in soil. It also synthesizes and secretes plant growth substances like, thiamin, riboflavin, pyridoxine, nicotinic acid, IAA (Indole Acetic Acid), gibberellins and vitamin B in addition to the production of antifungal antibiotics, which inhibit harmful fungi and have beneficial effect on crop growth. It also increases water uptake by plant. On the other hand, PSB enhances availability of phosphorus and promote root growth. It also secrete organic acids *viz.*, formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids, vitamins and growth promoting substances like IAA and gibberellins which might helped in better plant growth (Reddy, 2008). KSB enhance availability of potash and promote stem growth. The results of present study are in close conformity with findings of Godse *et al*. (2006) [10] and Dongardive *et al*. (2007) [7] in gladiolus; Satyavir (2007) [23] and Pandhare *et al*. (2009) [17] in tuberose; Parmar (2006) [18] and Singh (2007) [26] in rose; Lele *et al*. (2009) [14] in gerbera; Khan *et al*. (2009) [12] in tulip.

Table 1.1

The data revealed that the maximum number of tiller per plant (2.40), fresh weight of plant (129.35 g), dry weight of plant (44.28 g) and minimum days taken for opening of first spike (49.06) were registered with the combined application of humic acid 0.2% with *Azotobacter* @ 2.5 ml/plant + PSB @ 2.5 ml/plant + KSB @ 2.5 ml/plant (B_4F_3) in pooled, respectively. The results may be due to positive interactive effect of humic acid with biofertilizers. Humic acid is especially beneficial in freeing up nutrients in the soil so that they are made available to the plant as needed. For instance if an aluminum molecule is binded with a phosphorus one, humic acid detaches them making the phosphorus available for the plant. Humic acid is also especially important because of its ability to chelate micronutrients increasing their bio-availability. Humic substances (HS) are the major fraction of the soil organic matter which represents the final stage of a complex interaction between non-living organic matter and microbial communities. Humic acid and biofertilizer have great influence on the production of gladiolus crop. Humic acid and biofertilizer improve the ability of soil to solubilize phosphorus, potash and nitrogen for better crop production. Different characteristics of maize were increased in the treatments where different concentration of PSB biofertilizer applied with humic acid. The results of present study are in close conformity with findings of Bhalla *et al.* (2006) [2] and Pandey *et al.* (2013) [16] in gladiolus; Jadhav *et al*. (2014) [11] in marigold; Bihari *et al*. (2009) [4]. in rose and El-Shabrawy *et al*. (2010) [8] in cucumber Table 2.

Effect of Biostimulants

Significantly maximum flowering span (24.36 days), fresh weight of whole spike (51.68 g), dry weight of whole spike (23.41 g), length of spike (55.33 cm), longevity of spike (11.37 days) and vase life of spike (12.78 days) were also recorded with an application of humic acid 0.2% (B_4) in pooled. The results might be due to role of humic acid. By the application of humic substance to plants, the growing plants are supplied with food, its application also results in productive and fertile soil, which increases the water holding capacity of soil and increase quality of flower. Increase in the protein and mineral contents of most crops is possible by the application of humic substances. The improvement of soil properties resulted to better growth which also enhances the flowering parameters including flowering span, longevity as well as fresh and dry weight of spike. Humic acid might have increased the carbohydrate accumulation and which in turn would have hastened the spike emergence and produce a quality flower. Similar results were also obtained by Sankari *et al*. (2015) [22] in gladiolus; Khenizy *et al*. (2013) [13] in gerbera; Yasser *et al*. (2011) [29] in hibiscus; Ahmad ali *et al*. (2014) [1] in tulip; Vijayalakshmi and Mathan (1997) [28] in sunflower.

Effect of Biofertilizers

Significantly maximum flowering span (23.62 days), fresh weight of whole spike (50.38 g), dry weight of whole spike (22.72 g), length of spike (54.32 cm), longevity of spike (10.99 days) and vase life of spike (12.39 days) were registered with an application of *Azotobacter* @ 2.5 ml/plant + PSB @ 2.5 ml/plant + KSB @ 2.5 ml/plant (F_3) in pooled. This might be due to possible role of *Azotobacter,* PSB and KSB. The earliness of bud initiation in biofertilizer-inoculated plants may be attributed to easy uptake of nutrients and simultaneous transport of growth promoting substances like cytokinin to the axillary buds, resulting in breakage of apical dominance. Ultimately, this has resulted in a better sink for faster mobilization of photosynthates and early transformation of plant from vegetative to reproductive phase. The increase in phosphorus is found to be involved in the initiation of flower primordial formation leading to increase in number of flower and size as well as the attributes of yield. The higher photosynthesis enhanced food accumulation, which might have resulted in better plant growth and subsequently higher number of flowers per plant. The improvement in individual flower weight might be because of the fact that the combined application of various biofertilizers improved the soil health, water retention capacity and availability of microorganisms. The improvement in flowering duration might be because of the fact that the combined application of bio-fertilizers improved the soil health availability of microorganisms. The results of present study are in close conformity with findings of Gangadharan and Gopinath (2000) [9], Godse *et al.* (2006) [10] and Dalve *et al*. (2009) [6] in gladiolus; Chopde *et al.* (2007) [5] and Shankar *et al*. (2010) [25] in tuberose; Khan *et al*. (2009) [12] in tulip; Bhalla *et al*. (2007) [3] in carnation.

Table 2.1

Significantly maximum flowering span (25.66 days), fresh weight of whole spike (56.21 g), dry weight of whole spike (25.76 g), length of spike (59.52 cm), longevity of spike (11.79 days) and vase life of spike (13.44 days) were recorded with an application of humic acid 0.2% with *Azotobacter* @ 2.5 ml/plant + PSB @ 2.5 ml/plant + KSB @ 2.5 ml/plant (B_4F_3) in pooled. There is positive interactive effect of humic acid with various biofertilizers as they increase their use efficiency which resulted to better performance of majority of parameters. By the application of humic acid with biofertilizers to plants, the growing plants are supplied with food, its application also results in productive and fertile soil, which increases the water holding capacity of soil and increase quality of flower. Increase in the protein and mineral contents of most crops is possible by the application of humic substances. Combine application of humic acid with biofertilizers increased the concentration of macro and micro nutrients in the soil which ultimately resulted in vigorous growth of plants with appreciable flower diameter. The improvement in individual flower weight might be because of the fact that the combined application of biostimulants and biofertilizers in which humic acid increased availability of biofertilizers and make available nutrients for plant. The improvement in flowering duration might be because of the fact that the combined application of bio-fertilizers and biostimulants improved the soil health, water retention capacity and availability of microorganisms. The results of present study are in close conformity with findings of Bhalla *et al.* (2006) [2], Pandey *et al.* (2013) [16]. in gladiolus; Jadhav *et al*. (2014) [11]. in marigold and Bihari *et al*. (2009) [4] in rose.

Summary and Conclusion

From the foregoing discussion it can be concluded that for getting higher vegetative growth, flowering and quality in gladiolus cv. American Beauty should be sprayed with combined application of humic acid 0.2% with *Azotobacter* @ 2.5 ml/plant + PSB @ 2.5 ml/plant + KSB @ 2.5 ml/plant at first spray at 30 DAP, second spray at 45 DAP and third spray at 60 DAP of biostimulants (humic acid 0.2%) and soil application of biofertilizers (*Azotobacter* @ 2.5 ml/plant +

PSB @ 2.5 ml/plant + KSB @ 2.5 ml/plant) at the time of planting and two month after planting.

Table 1: Effect of biostimulants and biofertilizers on growth parameters in gladiolus (Pooled)

Treatments	Plant height (cm) at 90 DAP	Number of leaves per plant	Number of tillers per plant	Fresh weight of plant (g)	Dry weight of plant (g)	Days taken for opening of 1st spike (days)
Biostimulants (B)						
B_1 - Banana pseudostem Sap 1%	104.78	7.18	1.81	117.50	29.19	60.67
B_2 - Seaweed extract 1%	102.90	7.04	1.53	113.71	23.28	63.82
B_3 - Panchgavya 3%	108.90	7.50	1.93	126.54	32.25	56.54
B_4 - Humic acid 0.2%	113.01	7.66	2.06	131.38	38.28	55.41
S.Em. ±	1.20	0.07	0.02	0.80	0.39	1.09
C.D. at 5%	3.42	0.20	0.07	2.28	1.10	3.10
Biofertilizers (F)						
F_1 - *Azoto*. + PSB + KSB each @ 1.5 ml/plant	103.73	7.14	1.65	116.70	25.60	62.86
F_2 - *Azoto*. + PSB + KSB each @ 2.0 ml/plant	107.57	7.32	1.79	122.23	30.22	58.52
F_3 - *Azoto*. + PSB + KSB each @ 2.5 ml/plant	110.89	7.58	2.06	127.91	36.43	55.94
S.Em. ±	1.04	0.06	0.02	0.70	0.34	0.94
C.D. at 5%	2.96	0.18	0.06	1.98	0.95	2.68
Interaction (B X F)						
S.Em. ±	2.08	0.12	0.04	1.39	0.67	1.89
C.D. at 5%	NS	NS	0.12	3.96	1.91	5.37
CV%	4.75	4.14	5.64	2.79	5.35	7.83

Table 1.1: Interaction effect of biostimulants and biofertilizers on growth parameters in gladiolus (Pooled)

Treatment combinations	Number of tillers per plant	Fresh weight of plant (g)	Dry weight of plant (g)	Days taken for opening of 1st spike (days)
B_1F_1	1.68	111.50	25.62	67.46
B_1F_2	1.84	118.54	29.42	60.04
B_1F_3	1.91	122.47	32.54	54.50
B_2F_1	1.41	104.64	19.91	60.61
B_2F_2	1.55	111.85	23.52	60.67
B_2F_3	1.61	124.63	26.42	70.18
B_3F_1	1.66	123.80	23.99	59.25
B_3F_2	1.84	126.43	30.28	56.96
B_3F_3	2.29	129.39	42.48	50.03
B_4F_1	1.87	126.87	32.88	64.11
B_4F_2	1.92	132.10	37.67	56.43
B_4F_3	2.40	135.17	44.28	49.06
S.Em. ±	0.04	1.39	0.67	1.89
C.D. at 5%	0.12	3.96	1.91	5.37
CV%	5.64	2.79	5.35	7.83

Table 2: Effect of biostimulants and biofertilizers on flowering and quality parameters in gladiolus (Pooled)

Treatments	Flowering span (days)	Fresh weight of whole spike (g)	Dry weight of whole spike (g)	Length of spike (cm)	Longevity of spike (days)	Vase life of spike (days)
Biostimulants (B)						
B_1 - Banana pseudostem Sap 1%	20.77	45.38	19.34	50.37	10.05	11.23
B_2 - Seaweed extract 1%	20.00	42.88	17.66	48.94	9.38	10.58
B_3 - Panchgavya 3%	22.11	48.20	22.01	54.02	10.51	12.29
B_4 - Humic acid 0.2%	24.36	51.68	23.41	55.33	11.37	12.78
S.Em. ±	0.25	0.77	0.22	0.44	0.14	0.14
C.D. at 5%	0.72	2.19	0.62	1.25	0.40	0.41
Biofertilizers (F)						
F_1 - *Azoto*. + PSB + KSB each @ 1.5 ml/plant	19.87	42.63	17.61	49.75	9.54	10.96
F_2 - *Azoto*. + PSB + KSB each @ 2.0 ml/plant	21.93	48.10	21.48	52.42	10.45	11.82
F_3 - *Azoto*. + PSB + KSB each @ 2.5 ml/plant	23.62	50.38	22.72	54.32	10.99	12.39
S.Em. ±	0.22	0.67	0.19	0.38	0.12	0.12
C.D. at 5%	0.63	1.90	0.53	1.08	0.34	0.36
Interaction (B X F)						
S.Em. ±	0.44	1.34	0.38	0.76	0.24	0.25
C.D. at 5%	1.25	3.80	1.07	2.17	0.69	0.71
CV%	4.94	6.95	4.47	3.58	5.75	5.22

Table 2.1: Interaction effect of biostimulants and biofertilizers on flowering and quality parameters in gladiolus (Pooled)

Treatment combinations	Flowering span (days)	Fresh weight of whole spike (g)	Dry weight of whole spike (g)	Length of spike (cm)	Longevity of spike (days)	Vase life of spike (days)
B_1F_1	18.49	40.12	15.99	47.50	9.55	11.07
B_1F_2	20.74	43.73	19.75	49.76	10.22	10.98
B_1F_3	23.09	52.31	22.29	53.84	10.36	11.65
B_2F_1	19.25	37.89	14.28	46.60	8.52	9.81
B_2F_2	19.03	49.01	20.16	50.94	9.09	10.63
B_2F_3	21.72	41.75	18.53	49.27	10.53	11.31
B_3F_1	18.80	44.73	19.74	52.03	8.76	10.40
B_3F_2	23.51	48.61	21.98	55.37	10.99	13.32
B_3F_3	24.03	51.26	24.29	54.66	11.28	13.15
B_4F_1	22.96	47.80	20.44	52.85	10.83	12.57
B_4F_2	24.46	51.04	24.04	53.62	11.01	12.34
B_4F_3	25.66	56.21	25.76	59.52	11.79	13.44
S.Em. ±	0.44	1.34	0.38	0.76	0.24	0.25
C.D. at 5%	1.25	3.80	1.07	2.17	0.69	0.71
CV%	4.94	6.95	4.47	3.58	5.75	5.22

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