

HUMIC ACID APPLICATION IMPROVES THE GROWTH, FLORET AND BULB INDICES OF GLADIOLUS (*Gladiolus grandiflorus* L.)

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ABSTRACT: Humic acid is an organic acid having positive effect on plant growth. The present study was carried out to see the effect of humic acid in combination with NPK on soil for growth, flower production and vase life of gladiolus flowers. Five treatments *i.e.* nitrogen, phosphorous, potash (NPK) (17:17:17) (10 g), 8% humic acid (1.0 mL) + NPK (17:17:17) (10 g), 8% humic acid (2.0 mL) + NPK (17:17:17) (10 g), 8% humic acid (3.0 mL) + NPK (17:17:17) (10g) were studied, whereas treatment without exogenous feeding of NPK and humic acid served as control. All the treatments were applied at plating and 3rd leaf stage through soil. All the treatments were arranged in randomized complete block design (RCBD) in triplicate. Results depicted that humic acid (3.0 mL) in combination with NPK (17:17:17) produced higher sprouting, plant height, number of leaves per plant, leaf area, spike length, spike diameter, stem length, flower characteristics, vase life, number of cormels plant⁻¹, diameter of cormels and weight of cormels per plant than all other treatments. However, plants grown without application of humic acid (only NPK) showed relatively less and least growth characteristics and poor floral and bulb indices as compared to other treatments. It could be concluded that humic acid application can increase the vase life by increasing growth indices such as leaf area, fresh and dry weight of floret.

Keywords: Cut flower, gladiolus, growth, humic acid, nutrition..

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INTRODUCTION

Gladiolus (*Gladiolus grandiflorus* L.) is a bulbous plant which is commercially cultivated as cut flower throughout the world. Due to its excellent vase life and captivating colors, it has great economic value in global trade in landscaping (Bose *et al.*, 2003). The production demand of cut flowers has accelerated manifolds in the last decade and is expected to spring up further around the world. In Pakistan, commercial flowers cultivation is curtailed to limited areas. Nonetheless, recently cut flower production has increased and this increase is estimated to continue because of the high demand of gladiolus flowers. Gladiolus yield in Pakistan is low due to improper fertilizer use (Riaz *et al.*, 2007). Imbalance fertilization may reduce gladiolus flowers production and may also result in soil and environmental pollution. Gladiolus productivity hinges upon the amounts, forms and frequency of application of plant nutrients and also application of some organic acids (Zafar, 2007).

Soil health is important factor for the production of horticultural crops. Reduction in soil health and microbial activity results in decreased plant growth and poor crop stand (Baldotto and Baldotto, 2013). Due to

rapid increase in its flower demand, there is a need to increase the production of cut flower with less environmental disturbance. This could be possible by the integrated use of organic and inorganic sources of nutrients.

Humic acid is an organic acid that has been reported to promote plant growth by acting as a plant growth regulator and exertion of hormone-like activities (Mora *et al.*, 2010 and Arancon *et al.*, 2006). The promotion of photosynthesis and respiration has also been reported by Heil (2005) and chlorophyll content by Xu *et al.* (2012). Nikbakht *et al.* (2008) found that humic acid application increases the flower quality and diameter, plant height, and vase life in Gebera cut flower by restricting the growth of pathogenic bacteria. The studies have shown that humic acid may increase the nutrient absorption, photosynthetic activity, hence improve the quality and yield of the ornamental crops (Fan *et al.*, 2015). Contrarily, chemical fertilizers like NPK play an important role in achieving high yield. As a consequence, the use of humic substances has often proposed as a method to improve crop production. In a study Woltz (2001) stated that chemical fertilizer application in gladiolus improves growth rate and increases the flower size. Nitrogen is a vital plant nutrient

which is involved in the preparation of organic molecules like nucleic acid, protein and enzymes. Butt (2005) reported that by increasing the nitrogen supply, number of flowers, corms and cormels increased. Phosphorous (P) is also important for the health of plants as it requires in less quantity as compared to nitrogen. Potassium (K) enhances root growth, enzyme activity and controls the turgidity. Potassium decreases energy loss, respiration and is involved in protein synthesis thus increases the quality and vase life of flowers (Rajiv and Misra, 2000). The response of humic acid on plant growth has been reported in a wide range of plant species. However, no information is available regarding the application of humic acid on gladiolus flower yield and quality so far. There is a need to gain insight regarding the effect of humic acid application on grain, floret production and vase life of gladiolus flowers. The application of humic acid in combination with NPK may increase the gladiolus production more sustainably as compared to alone NPK application or excessive NPK application. Therefore, the current study was conducted to examine the effect of humic acid in combination with NPK fertilizer on the growth, flower and corm characteristics of gladiolus cut flowers.

MATERIALS AND METHODS

An experiment was conducted during 2013 at Floriculture Research Area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan (31°25'N, 73°04'E). The planting material was bought from Green works Pvt. Ltd., Lahore, while humic acid was obtained from Institute of Soil and Environment Sciences, University of Agriculture, Faisalabad. Humic acid was extracted from a rock material 'Leonardite' with 5.5-6.0 pH. Experimental soil consisted of 0.659% organic matter, 11.57% available N, 9.6 mg kg⁻¹ available P, 204 mg kg⁻¹ available K, 2.47 dSm⁻¹ EC and 8.7 pH. The corms of 3-4 cm diameter were stored at room temperature before plantation and corms were planted in mid-November on 60 cm apart ridges with 20 cm interplant distance. There were five treatments; control (nothing was applied), NPK (17:17:17) (10 g), 8% humic acid (1.0 mL) + NPK (17:17:17) (10 g), 8% humic acid (2.0 mL) + NPK (17:17:17) (10 g), 8% humic acid (3.0 mL) + NPK (17:17:17) (10 g). Five treatments with 50 plants in each including control were studied in the experiment. All the treatments were applied two times by soil application method, first at the time of planting and 2nd at three leaf stage. The treatments were arranged in randomized complete block design (RCBD) with three replications. The *Gladiolus grandiflorus* cv. White prosperity was obtained from "Greenworks Pvt. Limited, Lahore" an importer of "Stoop Flower Bulb Company of Holland" in Pakistan.

Growth characteristics: The parameters regarding growth characteristics were days to sprouting, sprouting percentage, plant height (cm), number of leaves per plant, leaf area (cm²) and days to flower emergence. Plant height and number of leaves per plant were measured after 60 and 70 days of sowing respectively. Leaf area was measured by portable leaf area meter. Days to sprouting were calculated from time of planting to seedling emergence. Sprouting percentage was calculated when all corms were open by the formula as given below

$$\text{Sprouting percentage (\%)} = \frac{\text{Total number of corms sprouted}}{\text{Total number of corms sown}} \times 100$$

Flower Indices: The parameters regarding flower characteristics were spike length (cm), spike diameter (cm), stem length (cm), days to flower opening, number of floret plant⁻¹, fresh and dry weight of floret (g) and vase life (days). Measuring tape and digital caliper was used to measure the spike length, stem length and spike diameter respectively. Five samples were collected from each treatment for measuring fresh weight of floret then samples were kept in oven at 72°C for 72 hours to measure the dry weight of floret. For vase life evaluation, stems were harvested in the morning before 10:00 h, leaves were trimmed, and stems were tying on stakes taken to the post harvest evaluation performed in a laboratory within 1 h of harvest. On arrival, stems were sorted on the basis of stem diameter and number of open florets into groups of 10 stems, recut from bases to a uniform length of 45 cm, labeled, and were placed individually in vases containing 300 mL distilled water. The stems were arranged on benches at 25 ± 2 °C and 60 ± 10% relative humidity (RH) with 12:12 h photoperiod provided by cool-white florescent lamps. Stems were examined daily for their visual appeal and were considered dead, when more than half of florets were wilted, faded or dried (Ahmad *et al.*, 2011)

Bulb Indices: The parameters for bulb indices such as number of cormels plant⁻¹, diameter of cormels (cm) and weight of cormels per plant were measured. Diameter of cormels was measured with the help of vernier caliper.

Statistical analysis: The analysis was duplicated and the average value from each duplicate was used for statistical analysis. Data was analyzed following analysis of variance using Statistix 8.1 (Analytical Software, Tallahassee, FL, USA) software. The mean differences between treatments were observed using least significance difference (LSD) test at 0.05 probability level.

RESULTS

Growth Characteristics: Gladiolus growth characteristics were significantly affected by different

treatments of humic acid Table-1. Among different humic acid applications, the treatments having 3 mL humic acid application sprouted earlier as compared to control. Maximum sprouting percentage (100%) was observed under humic acid (3 mL) + NPK application while plants showed 70% sprouting under control while, humic acid application reduced days to complete maximum sprouting as compared to control. Additionally, humic acid (3 mL) + NPK showed significantly higher plant height (16.5%), leaf area (29.1%) as compared to the control. Other two humic acid treatments (1 mL and 2 mL) increased growth with relatively lower magnitude as compared to humic acid application (3 mL) along with NPK. Spike length and spike diameter was highest in response to humic acid (3 mL) application along with NPK while control treatment showed lowest spike length and spike diameter. Two application of humic acid with NPK produced longer stem (16.66%) as compared to untreated plants Table-1.

Floral Characteristics: Humic acid significantly affected the floral characteristics of gladiolus. The results indicated that gladiolus plants showed earlier flower emergence (100 days) and flower opening (119.33 days) when treated with humic acid (3 mL) along with NPK application, while untreated plants (control) completed flower emergence and flower opening in 103.33 and

123.67 days respectively Table-2. The humic acid application significantly increased number of florets per plant (18.92%) as compared to alone NPK application or control. Furthermore, fresh and dry weights of gladiolus flowers in humic acid treatment (3 mL) along with NPK were the greatest among other humic acid and along NPK as compared to control Table-2. The vase life was (27.77%) and (19.04%) greater than that of the control and NPK treatments respectively, and significant difference was observed ($P < 0.05$) under humic acid treatment (3 mL) along with NPK. The result showed that the vase life of gladiolus flowers was extended by being treated with humic acid Table-2.

Bulb Indices: Corm characteristics of the gladiolus were significantly affected under humic acid application Table-3. The cormels diameter and cormels weight increased with the increasing humic acid concentration which ranged from 1 mL to 3 mL along with NPK application. Humic acid application of 3 mL along with NPK increased cormels diameter (37.9%) and cormels weight (100%) as compared to control. Further, maximum numbers of cormels per plant (65%) were produced under humic acid and NPK while minimum were observed in control treatment Table-3. The results showed that humic acid application improved bulb characteristics of gladiolus flowers.

Table 1. Effect of humic acid on growth and development of *Gladiolus grandifloras*.

Treatments	Days to sprouting	Sprouting Percentage	Plant height (cm)	Number of Leaves Plant ⁻¹	Leaf area (cm ²)	Spike length (cm)	Spike diameter (cm)	Stem length (cm)
Control	9.33a	70.00 d	78.50 c	4.50 c	130.36 c	33.00 d	0.400 d	113.00 c
NPK (17:17:17)	8.66ab	75.00 cd	84.33bc	5.00 bc	133.71c	35.00 cd	0.456 cd	121.50 bc
HM (1 mL) + NPK	8.33abc	83.33bc	85.66ab	5.50 bc	136.18bc	36.66 c	0.521 bc	122.33 b
HM (2 mL) + NPK	7.33bc	91.67ab	87.33ab	6.00 ab	161.67ab	40.00 b	0.570 ab	123.83 ab
HM (3 mL) + NPK	6.66c	100.0a	91.50 a	6.83 a	168.36a	45.00 a	0.623 a	131.83 a
LSD (p≤0.05)	1.93	9.72	5.90	1.26	26.52	2.31	0.081	9.024

Values (means of three replicates) sharing letters in common within a column don't differ significantly at P<0.05. HM: Humic acid

Table 2. Effect of humic acid on days to flower emergence, days to flower opening, florets per plant, fresh and dry weight of floret as well as vase life of *Gladiolus grandifloras*.

Treatments	Days to Flower Emergence	Days to flower opening	Number of florets per plant	Fresh weight of floret (g)	Dry weight of floret (g)	Vase life (days)
Control	103.33 a	123.67 a	9.67 c	4.43 d	0.350 c	9.00 c
NPK (17:17:17)	102.33 ab	121.67 ab	10.00 c	4.86 cd	0.383 bc	9.66 bc
HM (1 mL) + NPK	101.67 bc	121.33 bc	10.33 bc	5.23 bc	0.450 bc	10.00 bc
HM (2 mL) + NPK	101.33 bc	120.00 bc	11.00 ab	5.56 ab	0.483ab	10.50 ab
HM (3 mL) + NPK	100.67 c	119.33 c	11.50 a	6.10 a	0.576 a	11.50 a
LSD (p≤0.05)	1.51	2.17	0.97	0.57	0.10	1.08

Values (means of three replicates) sharing letters in common within a column don't differ significantly at P<0.05. HM: Humic acid

Table 3. Effect of humic acid on bulb characteristics of *Gladiolus grandifloras*.

Treatments	Number of cormels clump ⁻¹	Diameters of cormels (cm)	Weight of cormels clump ⁻¹ (g)
Control	64.67 c	0.733 c	13.70 c
NPK (17:17:17)	75.50 bc	0.791 bc	15.36 bc
HM (1 mL) + NPK	84.67abc	0.833 bc	18.30 bc
HM (2 mL) + NPK	92.67 ab	0.883 b	22.48 ab
HM (3 mL) + NPK	106.83 a	1.011 a	27.45 a
LSD (P<0.05)	23.61	0.116	7.35

Values (means of three replicates) sharing letters in common within a column don't differ significantly at P<0.05. HM: Humic acid

DISCUSSION

The humic acid significantly improved the performance of gladiolus plants. Among humic acid treatments, application of 3 mL humic acid along with NPK resulted in the higher growth and development and vase life of gladiolus flowers. The relative increase in plant height, sprouting percentage, and leaf area was 16.5%, 42% and 29.1% respectively, for 3 mL application of humic acid along with NPK as compared to control. Similar results were reported by Atiyeh *et al.* (2002) who found positive effect of humic acid on the growth of cucumber and tomato. The increase in sprouting in the present study suggested that humic acid played a considerable role in sprouting or germination as earlier simulation due to humic molecules. Similar findings have been reported by Saleem *et al.* (2013) for the early sprouting in ‘Fado’ gladiolus. Further the difference between days to sprouting between humic acid treatment and alone NPK or control might be due to different developmental speed. Similar effects were also observed in the germination of maize where plant growth was accelerated and has been reported by Eyheraguibel *et al.* (2008). The increase in plant growth due to humic acid application was also reported previously by Celik *et al.* (2008) and Taha *et al.* (2006). Humic acid application also increased the number of fruits and/or flowers, leaf area and plant height in wheat as reported by Chen *et al.* (2004). In a study, Lulakis and Petsas (1995) found that humic acid was absorbed through roots and was translocated in other parts and increased the plant growth. The stem length of gladiolus also increased under humic acid application. Arancon *et al.* (2003) reported that different concentrations of humic acid increased the stem length and thickness of stem of marigold with various magnitudes as compared with control. It was also observed in this study that leaf area significantly increased due to humic acid application.

The humic acid might lead to induce significant improvements in leaf water balance, leaf water potential, chlorophyll contents and photosynthetic rates which would result in increased photosynthesis and leaf growth and development. Higher leaf area resulted in higher rate of photosynthetic rate, which ultimately resulted in higher growth rate and better photosynthates translocation. These results were similar to Baldotto and Baldotto (2013) who observed greater number of leaves due to increased uptake of micro and macronutrient and reduced water evaporation from soils. Furthermore, humic substances increase the uptake of nutrients, thus improve the wide range of nutrient dependent physiological processes and morphological development. Higher gladiolus growth due to humic acid application could also be due to stimulation of N metabolism and photosynthetic activity, increased leaf area and ultimately higher yield (Haghighi *et al.*, 2012). It was reported by Valdrighi *et al.*

(1996) that enhancement in the growth of tomato (*Solanum lycopersicum* L.) and cucumber leaves may be possible because of incorporation of humic acid into the soilless container medium which increased nutrient uptake by the plants.

It was observed that humic acid not only improved vegetative growth but also increased the number of florets per spike, florets fresh weight and dry weight Table-2. The results of present study are in line with the findings of Baldotto *et al.* (2013), Nikbakht *et al.* (2008) and Kaya (2005) who observed similar results in gerbera, gladiolus and common bean when treated with higher concentration of humic acid. Moreover, Canellas *et al.* (2008) found an extrusion of organic acid from maize roots in the rhizosphere, which when in contact with acidified environment in rhizosphere resulted in the breakdown of humic compounds. The product of breakdown of humic compounds could alter the metabolism at cellular level by the H^+ -ATPases in the plasma membrane of root cells (Canellas *et al.*, 2000). These mechanisms lead to acidification of the apoplast and the activation of cell wall degrading exo-enzymes, making it more susceptible to the vacuolar turgor pressure and consequently, to cell elongation. This may explain the effect promoted by humic acid increasing the growth and providing a greater and earlier flowering of *Gladiolus* (Baldotto and Baldotto, 2013). Similar findings were observed, in a study with higher number of florets per plant, floret fresh weight, dry weight, and vase life of gladiolus flowers under humic acid treatment as compared with alone NPK or control Table-2. Humic acid application along with NPK increased number of florets per plant as compared to alone NPK application. This could be associated with higher uptake of N, P and K nutrient from soil due to chelating action of humic acid, which resulted in development of more number of florets per plant. Further, Ricardo *et al.* (1993) reported earlier flowering and higher yield in marigold under humic acid application. Shortening crop cycle by early production not only lowers production cost and increases per unit return and profitability, but also lowers the risk of crop damage by different biotic and abiotic stresses. Humic acid application also improved the vase life of gladiolus flowers. One of mechanisms proposed to account for the enhancement in vase life showed a positive relation between enhanced vase life and higher accumulation of carbohydrates, higher flower fresh weight (Gul *et al.*, 2012 and Chutichudet *et al.*, 2011). Further, Fan *et al.* (2015) found that foliar application of humic acid on chrysanthemum increased flower size and fresh weight and delayed the loss in flower size and fresh weight in cut chrysanthemum flowers due to higher contents of soluble sugar and soluble protein in the leaves, which resulted in higher vase life. Similar results were found in this study, where humic acid application resulted in higher number of florets per plant with higher

fresh and dry weight and higher vase life as compared to control Table-2. In Gerbera, Nikbakht *et al.* (2008) found prolonged vase life when treated with humic acid at 1000 mg L⁻¹. Humic acid also had auxin like activity which enhanced nutrient uptake thus increased vase life of cut stems (Baldotto and Baldotto, 2013). Similar results were also explained by Ahmad *et al.* (2013) who substantiated that plants grown without humic acid and NPK application (control) or application of NPK alone, applied at planting, had poor growth, reduced yield, and inferior quality.

The present study revealed that, cormels production of gladiolus was also observed which found to be higher due to humic acid application along with NPK application. Further cormels were found to be much properly developed as examined by cormels diameter and cormels weight per plant, which may be due to healthy soil conditions due to humic acid application. The results are in agreement Baldotto and Baldotto (2013) who observed an increased number of cormels per plant, cormel diameter and cormel weight per plant might be due the effects of humic acid to make more mineral nutrients available to plants Table-3. Conclusively, improved performance of gladiolus flower and can be used to increase gladiolus flower production on large scale.

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