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Combined Effect of Humic Acid and NPK on Growth and Flower Development of *Tulipa gesneriana* **in Faisalabad**, **Pakistan**

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A research trial was conducted during 2011-2012 to elucidate the effects of humic acid (HA) and NPK to determine the optimum rate against different growth and floral attributes on tulip cultivar viz. 'Triumph'. The following five treatments T₀: (control), T₁: 10 g/m² NPK (17:17:17), T₂: HA $0.75 \text{ ml} (8\%) + 10 \text{ g/m}^2 \text{ NPK} (17:17:17), \text{ T}_3: \text{HA } 1.00 \text{ ml} (8\%) + 10 \text{ g/m}^2$ NPK (17:17:17) and T₄: HA 1.25 ml (8%) + 10 g/m₂ NPK (17:17:17) were applied under RCBD replicated thrice. All the vegetative and reproductive attributes were significantly influenced by the addition of humic acid and NPK and obtained results revealed that treatment T₄ was the most effective one compared with the other treatments. This treatment gave the inimitable outcomes concerning earliest sprouting and flowering, plant height increment, leaf area expansion, stem diameter, leaf chlorophyll contents, stalk length, vase life, fresh and dry flower biomass. It also increased the nutrient contents comparing with the T₃ and T₂. In comparison, the plants grown without HA and NPK application (control) followed by a single application of NPK (T₁) exhibited poor growth with reduced yield of inferior quality. The data obtained in this study conveyed that HA (1.25 ml of 8% humic acid) along with NPK application (10 g/m² NPK) helps to improve the uniform crop stand, plant growth and flower quality of tulip.

Keywords: Flower quality, Humic acid, Nutrient uptake, Plant growth, Tulip cut flower.

Abstract

INTRODUCTION

Tulips are one of the most popular springs of all time and ranked third most popular flowers world-wide next to rose and chrysanthemum in the cut flower trade (Van, 1999). The Netherland has prominent status by largest bulb grower and exporter in the world with about 65% of the total sales of flowers and plants (Schneider, 1991). Tulip is the top most liked flowering geophytes' of the Netherland, the largest area under any true bulb crop in the world is that of Tulipa, followed by *Narcissus, Iris, Hyacinthus* and *Lilium* (Khan *et al.*, 2006).

Production of cut flowers in Pakistan is estimated at about 10-12 thousand tons per annum (Khan, 2011) and Pakistan has tremendous potential for cultivation of floral crops on commercial scale due to availability of favorable soil climate and geographic location (Manzoor *et al.*, 2001). Nature gives the unique advantage to this country is the seasonal diversity in producing and supplying the best quality of cut tulip along with other flowers during the winter months of the European countries. This is just the time when a large part of Europe is subjected to frost, fog and snow causing reduction in the yield and production. Thus they have to depend largely on import to meet their domestic demand as most of spiritual and traditional festivals like Christmas, Valentine, Mother's Day and Happy New Year festivities are held in this period when the demand for flowers is at its peak. So, Pakistan has strong potential to earn foreign exchange with minimum cost of production by raising and improving the standards of our floricultural produce (Bukhari, 2005).

Humic based fertilizers and mineral contents are the excellent combination which provides the ideal environmental condition for plant growth and development (Mawgoud et al., 2007; Sara et al., 2010). The use of humic acid (HA) is a promising natural resource to be utilized as an alternative for increasing crop production. Humic acids make important contributions to improve soil stability, fertility, improves flower quality that lead to exceptional plant growth and micronutrient uptake (Knicker et al., 1993). Humic acid is an effective agent to use as a compliment to fertilizer which is mostly used for soil reclamation which reduces the harmful effects of synthetic fertilizers and some other chemicals from the soil. It also has the potential for the economization of water and fertilizers. Approximately 65-70% of organic matter in the soil mainly is derived from humic and fulvic acid substances (Khristeva, 1953). Zaghloul et al. (2009) examined the effect of potassium humate on vegetative growth and some chemical constituents on the seedlings of the popular ornamental plant Thuja orientalis L. Growth parameters like plant height, stem diameter, root length, fresh and dry weights of shoots and roots and NPK percentage content significantly affected by application of K-humate. Nikbakht et al. (2008) studied the exogenous effect of humic acid on plant growth, nutrient uptake, and vase life of Gerbera flower. Humic acid also improved NPK concentration and chlorophyll content in leaves, flower quality, flower diameter, flower longevity and plant height. Higher humic acid levels prolong the vase life of Gerbera flowers. Evans and Li (2003) studied the effect of humic acid on the growth of annual ornamental seedling like a pansy, marigold, geranium, vinca, and impatiens. All vegetative and floral parameters significantly improved by increasing the HA concentration and this was also concluded by Dore and Peacock (1997) that humic substances act as a soil conditioner for turf grass growth and improved root growth. As a consequence, the use of humic substances has often proposed as a method to improve crop production.

Keeping in view the importance of tulip in global cut flower, a research project was done to determine the potential of HA to affect growth, nutrient content and postharvest life of tulip.

MATERIALS AND METHODS

Research work was conducted in the green house, Rose Research Area, Institute of Horticulture Sciences, University of Agriculture, Faisalabad 2011-2012 (latitude 31°30 N, longitude 73°10 E and altitude 213 m) in order to elucidate the effect of soil based humic acid and NPK application on growth, yield and quality of cut *Tulipa gesneriana* L. viz. Triumph. Five treatments plan with three replications was designed in this experiment.

Treatments

Control T ₀		
10 g/m ² NPK (17:17:17)	(2 Applications)	T_1
0.75 ml of 8% HUMIC ACID + 10 g/m ² NPK (17:17:17)	(2 Applications)	T_2
1.00 ml of 8% HUMIC ACID + 10 g/m ² NPK (17:17:17)	(2 Applications)	T ₃
1.25 ml of 8% HUMIC ACID + 10 g/m ² NPK (17:17:17)	(2 Applications)	T_4

The treatments were laid out following Randomized Completely Block Design (RCBD). Before the start of the experiment, soil samples from various blocks of experimental field were collected and analyzed for various physio-chemical properties (Table 1). After sampling, the soil was thoroughly tilled, levelled and all cultural practices (weeding, plant protection measures, earthing up etc.) were done before plantation. Treatment materials (HA and NPK) were obtained from Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan extracted from organic residues and macronutrient combinations.

The planting material (corms) was obtained from "Green works Pvt. Limited, Lahore" an importer of "Stoop Flower Bulb Company, Holland". The corms stored at 4 ± 1 °C than taken out seven days before planting and kept in a laboratory at ambient temperature. These were planted during the end week of November, 2012 according to the layout of the experiment with 10 cm plant to plant distance on 60 cm spaced ridges. Seven corms were planted in each treatment and each treatment was replicated thrice with a total of 105 corms used in the study. The first application of humic acid and NPK fertilizers were applied at the time of planting bulbs, while the second dose was applied at 3-leaf stage. All other cultural practices like weeding, plant protection measures, staking, earthing up etc. were kept same for all treatments during entire period of study. Plants were allowed to grow and data regarding growth, flowering and corm indices were collected by using standard procedures.

Statistical analysis

The data pertaining to various parameters were analyzed by ANOVA techniques using Statistics 8.1® computer based software and significance of means was tested according to least significant difference test (LSD) at 5 % probability (Steel *et al.*, 1997).

RESULTS

Data regarding days to sprouting and sprouting percentage are presented in Table 2. The effect of HA on *Tulipa gesneriana* L. revealed highly significant difference ($P \le 0.05$) for days to sprouting (day) and germination percentage. Earliest sprouting (47.14 days) and maximum sprouting percentage (97.29%) was recorded in T₄ followed by all other treatments. For comparative analysis, statistical findings declared T₄ treatment as a best in which least days to sprouting were

experiment.					
Parameter	Unit	Value			
pHs		8.79			
ECe	dS m⁻¹	2.47			
Organic Matter	%	0.66			
Nitrogen	mg kg⁻¹	11.57			
Phosphorus	mg kg⁻¹	9.6			
Potassium	mg kg⁻¹	204			
Zinc	mg kg ⁻¹	1.9			

Table 1. Physical and chemical properties of soil use	d for
experiment	

Table 2. Effects of different humic acid levels on days to sprouting, sprouting percentage, plant height,
leaf area, stem diameter of tulip flower 'Triumph'.

Treatment	Sprouting days	Sprouting percentage	Plant height (cm)	Leaf area (cm²)	Stem diameter (mm)
To	58.43 ª	85.59 e	29.39 e	130.14 ^e	4.53 °
T_1	55.19 ^b	88.41 d	33.61 d	135.84 d	5.04 d
T_2	52.67 °	91.53 °	36.61 °	140.86 °	6.20°
Тз	49.81 d	94.67 ^b	38.15 ^b	145.28 ^b	8.28 b
T ₄	47.14 °	97.29 ª	40.27 ª	149.57 ª	10.06 ª

Means in columns followed by the same letters are not significantly different at $\alpha \leq 0.05$ level according to LSD test.

observed and T_0 took more time to sprouting among all other treatments. Same pattern was also observed in case of plant height where T_4 also gained dominance with maximum plant height of 40.27 cm followed by T_3 , T_2 , T_1 respectively, while the minimum plant height was found in T_0 (29.39 cm).

Significant effect was also observed in leaf area and stem diameter. Mean comparison of treatments depicted the superiority of T_4 over all the other treatments by giving maximum leaf area (149.57 cm²) and highest producing stem diameter (10.06 mm). In contrast, the least leaf area and stem diameter was observed in T_0 which are ranked in lowest order. These results revealed that treatments are highly significant with respect to leaf area and stem diameter of tulip statistically and these attributes increased with increase in humic acid application that advocated the importance of HA for stem diameter.

Data pertaining to stalk length and days to flower emergence (DAP) is presented in Table 3. Comparison of mean indicated the highly significant superiority of T_4 in stalk length (38.15 cm) with earliest flower induction in 123.66 days followed by the T_3 , T_2 , and T_1 and T_0 , respectively. Significant variation was also examined in vase life case and findings clearly confirmed the dominance of T_4 over other treatments for vase life (8.66 days) followed by T_3 , T_2 and T_1 . Least vase life (3.00 days) was seen in T_0 .

Mean values obtained for leaf chlorophyll contents (SPAD value) are presented in Table 3. The result indicated that soil application of humic acid along with macronutrient gave highly significant (p <0.05) results on leaf chlorophyll contents of Tulip. The highest value was achieved in T₄ (45.62 SPAD) with rest of other treatments. Data regarding tepal diameter revealed highly significant difference and highest tepal diameter (10.51 mm) was recorded in superior T₄.

The results in Fig. 1 showed clear significantly positive trend in fresh and dry biomass by increasing the concentration of humic acid along with NPK. Treatment T_4 demonstrated that the highest fresh weight (34.67 g) and dry weight (6.88 g) of flower which was statistically superior to other treatments. The lowest observation for both measured traits was recorded in control treatment.

It is evident from the data (Table 4) that all minerals content (N, P and K %) under investigation were gradually increased by increasing humic acid concentrations. The observation re-

Table 3. Effects of different humic acid levels on stalk length, days to flower emergence, vase life, leaf
chlorophyll contents, tepal diameter of tulip flower 'Triumph'.

Treatment	Stalk length (cm)	Days to flower emergence (DAP)	Vase life (days)	Leaf chlorophyll contents (SPAD)	Tepal diameter (mm)
To	31.54 °	136.73 ª	3.00 e	39.02 ^e	6.28 ^e
T_1	33.36 d	133.64 ^b	4.90 d	36.34 d	6.64 ^d
T ₂	35.69 °	127.42 °	5.71 °	40.76 °	7.33 °
T ₃	36.11 ^b	125.42 d	6.42 ^b	42.68 ^b	8.03 ^b
T ₄	38.15 ª	123.66 ^e	8.66 ª	45.62 a	10.51 ª

Means in columns followed by the same letters are not significantly different at $\alpha \leq 0.05$ level according to LSD test.

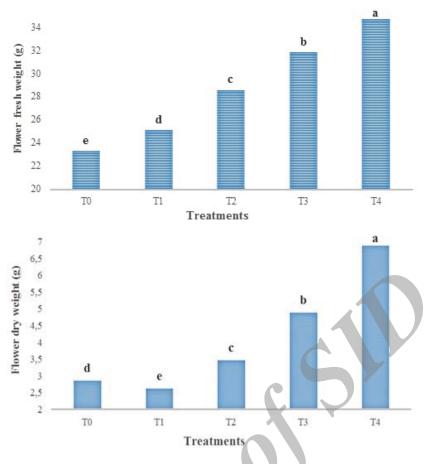


Fig. 1. The fresh and dry biomass of flowers of *Tulipa gesneriana* L. 'Triumph' as influenced by humic acid application.

garding the nutrient uptake was statistically analyzed and noted that T_4 has got the maximum nitrogen (5.97%), highest phosphorus contents (2.95%) and abundance available potassium contents (4.24%) followed by T_3 , $T_2 T_1$. The minimum values for these chemical constituents were recorded in T0 (control) which was calculated to be 3.23 % for N, 0.21% for P and 2.32 % for K, respectively.

DISCUSSION

This study focused on the response of tulip flower for different humic acid concentrations along with NPK to recognize the best treatment for maximum plant growth, nutrient uptake and vase life of flower. Significant role of humic acid and NPK was determined under greenhouse experiment in Faisalabad soil condition. The absolute findings of the experiment are in line with and verified by the findings of multiple researchers and are given as under categorically.

Treatment	Dry matter (%)	Leaf N contents (%)	Leaf P contents (%)	Leaf K contents (%)	
To	12.30 °	3.23 °	0.21 ^d	2.32 d	
T 1	10.45 d	3.70 d	0.84 °	2.52 °	
T ₂	12.14 °	4.40 °	1.15 °	2.76 °	
T ₃	15.40 ^b	5.23 ^b	2.01 ^b	3.00 b	
T ₄	19.84 ª	5.97 ª	2.95 ª	4.24 ^a	

Table 4. Effects of different humic acid levels on dry matter, N, P and K contents in leaves of tulip flower 'Triumph'.

Means in columns followed by the same letters are not significantly different at α <0.05 level according to LSD test.

Vegetative growth response

The ingredients for combined treatment of humic acid and NPK used in this research played a crucial role in plant growth and development of tulip. Concerned with the luminous efficacy of the humic acid and NPK on vegetative growth, Seadh *et al.*, 2012 concluded that the performance of plant growth parameters changed according to humic acid and the NPK application rate and the plants had better growth under maximum concentration. Early growth and the maximum germination percentage have significantly observed in our study when increasing the combined application of humic acid and NPK. These results are in line with the findings of Dixit and Kishore (1967) who reported that humic acid is growth promoter that makes the seeds possible to sprout earlier and reduce the mean germination time in a number of species including ornamental seeds. Miroslava (1962) also, found his findings in line with our results that sodium humate at a concentration of 100 mg l⁻¹ accelerates the uptake of water by the swelling seed during the initial stage of imbibition. The fact that the seed takes up a sufficient amount of water sooner make it possible for the activation of enzyme systems which ensure normal germination to take place. So, it might be the reason for the increased sprouting percentage.

In the lights of the results of this study, it was seen that plant height, leaf area and stem diameter increased gradually when increased humic acid concentration. These observations are in accordance with the findings of Turkmen *et al.*, 2004 who reported that there is a significance effect of different levels of humic acid (0, 500, 1000 and 2000 mg kg⁻¹) on plant height and vegetative growth for stem elongation that improved by increasing the HA concentration as per plant requirement. Basbag (2008) stated that plants provided with the highest dose of 150 ppm HA produce maximum leaf expansion. It was reported by Atiyeh *et al.* (2002) that there is improvement of nutrient uptake especially of nitrogen, phosphorous and sulfur and hormone by the activity of HA. These results may be due to the combined application of HA and macro nutrient helps to transport vital sugars through plant membranes and promotes proper cell division, cell wall formation and also acts as an enzyme activator in protein and hormones (Mahgoub *et al.*, 2010).

Stalk length is a well-known attribute in bulbous cut flowers. Our results revealed that stem length was increased with the increase in humic acid concentration that advocated the significance of humic acid. It was inferred from the above results that combination of HA and NPK effect more than their single application. This may be due to improvement of plant growth in terms of stem elongation by hormone like activity of HA. These results are in harmony with those of Turkmen *et al.* (2004) who reported the significant effect of different levels of HA on stem length improvement. This is well documented by many other researchers (Arancon *et al.*, 2003; Atiyeh *et al.*, 2002). The increase in stalk length might be due to the availability of better nutrition to the plant. Padem and Alan (1995) observed that combined NPK applications may elevate the nitrogen, potassium and phosphorus contents. The increase in stalk length might be due to elevated levels of macronutrients and humic acid which have a positive effect on floral characteristics.

Leaf chlorophyll contents (SPAD) response

It was concluded that among HA treatments, treatment containing HA and NPK application had more leaf chlorophyll contents as compared to rest of the treatments. Leaf chlorophyll contents were increased with an increase in the concentration of HA along with application of NPK which show that the treatments comprising the both HA and NPK have a significant effect on the leaf chlorophyll contents. The increase in chlorophyll content might be due to improvement in nitrogen assimilation from NPK (Minotti *et al.*, 1994). Regarding the beneficial effect of HA on photosynthetic pigments, may be due to its role in increasing the rates of photochemical reduction (Coco and Agnolla, 1988). These results are in agreement with Visser (1986) who reported that photosynthesis efficiency and chlorophyll contents were significantly increased in humus treated plants. These humic substances were able to support electron transport and to increase photosynthesis efficiency.

Reproductive growth response

In case of days to flower emergence, it was concluded from the research findings that days to flower emergence was decreased with increases HA application that described the vital role of HA played in the flower emergence reduction period. The result showed that the combination HA and NPK induced earlier flowering. This suggests that a combination of the above nutrients is the best due to high nutrients concentration. Balanced nutrition is one of the best practices to get early flowering in plants (Kumar and Haripriya, 2010). Furthermore, Ricardo et al., 1993 observed that the application of HA may lead to improved production of marigold bedding plants and earlier flowering. The application of the HA and nutrients increase the root portion of the plants which enhance rooting and growth performance of the bedding plants.

The vase life of tulip plants increased significantly by giving a maximum dose of humic acid treatment. Similar results were painted by Kulikova *et al.*, (2005) who reported that HAs have auxin-like activity that enhanced the nutrient uptake which may be responsible for the good floral growth. Khenizy *et al.* (2013) also, reported that HA plays vital role for maximum water uptake and then prolong the vase life in gerbera cut flowers.

Tepal diameter was also increased in that treatment which contained high dose of humic acid as in T₄. Combined effect of humic substances along with macro nutrients have a vital role in the growth and development of a strawberry plant, same results painted by Pilanali *et al.*, 2003.

Fresh and dry weight response

The increase in fresh weight of flower is measured as an important parameter by the reference of growth. The application of HA and NPK contents increases vegetative growth which in turn resulted in higher assimilate production (carbohydrates and proteins), which might have been utilized for better development (Shanmugam and Veeraputhran, 2001). The results revealed that treatments are highly significant with respect to fresh weight of the flower (g) of tulip and are statistically at par. Fresh weight of the flower (g) increased with an increase in the HA application that advocated the importance of HA for fresh weight of flower. The results of the present study are harmonious with those of Kauser *et al.* (1985) they reported that plants treated with HA and NPK showed more fresh weight of different crops and increased total carbohydrate.

The results revealed that treatments are highly significant with respect to the dry weight of flower of the tulip statistically and dry weight of flower increased with an increase in humic acid application due to more uptakes of nutrients as described by Azarmi *et al.* (2008). These results are similar to the research findings of Lee and Bartlett (1976) and Albuzio *et al.* (1994), they reported an increase in shoot dry weight of different crops by the application of humic substances. The results show that the treatments comprising the both HA and NPK have a significant effect on the dry weight of the plant. The results are agreed with the findings of Arnacon *et al.* (2003) who reported that increased dry matter production may be attributed to the combined application of HA and inorganic micronutrients. N have a greater effect on leaf photosynthetic rates which are directly associated with plant dry matter production. Phosphorus has participated in higher protein synthesis and may improve the vegetative growth and dry matter accumulation (Kumar *et al.*, 2003).

Nutrient uptake response

Humic acid promote the conversion of mineral nutrients into available forms for plants. Our findings claim that nutrient absorption by plants significantly higher if we increase the humic acid level along with NPK application. In T_4 treatment in which higher dose of humic acid exist; facilitate the plants leaves for maximum nutrient accumulations (NPK contents).

Nitrogen is primarily responsible for vegetative growth. Nitrogen assimilation into amino acids is the building block of protein in the plant. It is a component of chlorophyll and is required for several enzyme reactions. A similar trend has also been reported by Magda (2003) who elabo-

rated that nitrogen contents increased with increases HA and NPK application in squash plant. These results are also in the line with the findings of Chen and Aviad (1990), who reported significant differences in nitrogen uptake by leaves.

Phosphorus is a major component in plant DNA and RNA. Phosphorus is also critical in root development, crop maturity and seed production. A similar trend has also been reported by Shehata *et al.* (2011), they observed that HA plays a vital role in nutrient accumulation to roots and hence phosphorous absorption rate may be increased by HA application at critical growth stages.

Potassium increases water use efficiency and transforms sugars to starch in the grain-filling process. It's important for a plant's ability to withstand extreme cold and hot temperatures, drought and pests. The addition of "K" contents in plant leaf higher by increasing the HA and NPK application. Homogenous results showing the positive co-relationship between the doses of HA and the potassium contents of the leaves were observed by Nikbakht *et al.* (2008) and Chen *et al.* (2004).

CONCLUSION

This can be concluded from the research that the addition of humic acid along with macro nutrient mixes (NPK) had a positive effect on the growth of *Tulipa gesneriana* L. The magnificent role of HA in this study has revealed as a greatest growth enhancement tonic especially for the cut flower industry. The scientific findings regarding this particular field are very rare; however a conclusive clue has emerged in this experiment for further research. Plant growth gradually increased when increasing the concentration of HA. All the vegetative and floral growth produced vigorously without soil and environmental degradation. The potential exists for growers to use HA along with macro nutrients on a commercial scale in order to achieve the growth and flower quality in tulip.

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تاثیر تلفیق اسید هیومیک و NPK روی رشد و نمو گل Tulipa gesneriana در فیصلآباد پاکستان

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این مطالعه به منظور بررسی اثرات اسید هیومیک (HA) و NPK در سال ۲۰۱۲-۲۰۱۱ با هـدف تعيين بهتريـن نـرخ رشـد و گلدهـی روی گل لالـه رقـم 'تريومـف' انجـام شد. ۵ تیمار عبارتند از T۰ (شاهد)، T۱: ۱۰ گرم در متر مربع NPK (۱۷:۱۷:۱۷)، T۲: ۰/۷۵ میلیلیتر اسید هیومیک (۸٪) + ۱۰ گرم در متر مربع NPK (۱۷:۱۷:۱۷)، ۲۳: ۱ میلی لیتر اسید هیومیک (۸٪) + ۱۰ گرم درمترمربع NPK (۱۷:۱۷:۱۷) و ۲۴: ۱/۲۵ میلیلیــتر اسـیدهیومیک (۸٪)+ ۱۰ گـرم در مترمربـع NPK (۱۷:۱۷:۱۷) کـه در قالـب طرح RCBD با سه تکرار مورد بررسی قرار گرفتند. همه خصوصیات رویشی و زایشی به صورت معنی داری تحت تاثیر اسید هیومیک و NPK قرار گرفتند و نتایج حاصله نشان داد که تیـمار T۴ نسبت بـه سـایر تیمارهـا موثرتـر بـود. ایـن تيمار نتايج بىنظيرى از نظر زود گلدهي، افزايش ارتفاع گياه، توسعه سطح برگ، قطـر سـاقه، مقـدار کلروفیـل بـرگ، طـول سـاقه گلدهنـده، عمـر گلجایـی و وزن تـر و خشک گل به همراه داشت. ایـن تیـمار مقـدار مـواد غذایـی را نیـز در مقایسـه بـا ۲۲ و TT افزایش داد. گیاهان شاهد و بعد از آن گیاهان تیمار T۱ رشد ضعیفی داشته و عملکرد و کیفیت آنها کاهش یافت. نتایج حاصل از این مطالعه نشان داد که HA (۱/۲۵ میلیلیـتر اسـید هیومیـک ۸٪) همـراه بـا NPK (۱۰ گـرم در مترمربـع) بـه بهبود یکنواختی محصول، رشد گیاه و کیفیت گل لاله کمک میکند.

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