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Humic acid and folic acid application improve marketable traits of cut tuberose (*Polianthes tuberosa*)

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Abstract

Tuberose is an important cut flower in Iran and throughout the world. Since nutrition is of crucial significance for the growth, development, and quality of ornamental plants, the present study aimed to explore the effect of different levels of humic and folic acids on yield and quantitative and qualitative traits of tuberose (*Polianthes tuberosa* L.) based on a randomized complete block design with three replications in the Faculty of Agriculture, Agriculture and Natural Resources University of Gorgan, Iran in 2016. Humic and folic acids were applied at the rates of 0, 50, 100 or 150 mg L^{-1}. The foliar application was performed in three phases, i.e. 30, 50 and 70 days after planting. The recorded traits included spike length, stem length and diameter, shoot fresh weight, floret number, leaf number and area, flower emergence time, bulblet number, root development depth, N, P and K content, vase life, and total chlorophyll. Analysis of variance showed that the application of humic acid significantly influenced all measured traits, except leaf number and flower emergence time. As the humic acid rate was increased to 150 mg L^{-1}, leaf total chlorophyll content was increased by 52 percent showing a significant difference with control. The foliar application of humic and folic acids increased N, P and K contents of leaves, and the strongest effect was observed at the rate of 150 mg L^{-1}. The results lead us to the conclusion that the application of 150 mg L^{-1} humic acid and folic acid had the strongest impact on increasing the quantitative and qualitative traits of tuberose.

Keywords: Foliar application; Humic acid; Nitrogen, Total chlorophyll; Tuberose

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Introduction

Tuberose (*Polianthes tuberosa* L.) from the genus Polianthes and the family Agavaceae is an ornamental bulbous plant species native to Mexico. Tuberose is a flower of tropical and semi-tropical origin. The cut flowers of this plant species have a high potential for post-harvest longevity (Naz *et al.* 2012). Tuberoses are harvested when the lower florets have just been opened. The unopened florets rarely open after harvest and this limits the quality manifestation of this plant species. Although tuberose is an exporting flower, its commercial production is hindered by the incomplete opening of the florets along the flowering stem, the premature shedding or the lack of the development of terminal florets and the short longevity of the remaining florets (Nardi *et al.* 2002). Plant growth stimulators and regulators contribute to the better establishment of plants in soil, their longer preservation and the increase in their chlorophyll level, which enhances the competitiveness and survival of the plants (Bahrani 2015). Most micronutrients are fixed in alkaline soils and plant roots are unable to absorb them from the soils adequately (Cakmak, 2008). A method to supply the food requirements of the plants is their foliar feeding. As the advantage of this method, nutrients are provided to the plants fast and efficiently (Zhang *et al.* 2014). Recently, much attention has been directed to the application of natural substances to improve plant growth. In this respect, vitamins like folic acid have been shown to influence the growth, yield and quality of many plant species. These compounds have desirable effects on the uptake of free radicals or active oxygen and this, in turn, affects photosynthesis and respiration processes (Nardi 2002). Also, vitamins have auxin activity (Fardet *et al.* 2008). The most known vitamins are ascorbic acid (vitamin C) and folic acid (vitamin B_9) that are synthesized in most plant species and influence their growth and development. The desirable impact of various vitamins on the growth and production of crops has been buttressed by many researchers, e.g. Abd El-Naeem and Abd El-Hakim (2009) and Al-Qubaie (2012).

Folic acid was first isolated from spinach (*Spinacia oleracea* L.) in 1941. It was composed of pteridine nuclei, para-aminobenzoic acid and glutamic acid, which is a water-soluble vitamin B (Cossins 2000; Wang *et al.* 2008). On the other hand, folic acid (vitamin B_9) is one of the most prominent B complex vitamins with an especial role in biochemical reactions, such as the metabolism of amino acids and the synthesis of nucleic acids (Andrew *et al.* 2000). Folic acid enables plants to synthesize RNA that is a nucleic acid and conveys information from DNA to ribosomes where it contributes to the synthesis of the proteins in plants (Al-Qubaie 2012). Folic acid is a central cofactor for the carbon transfer reaction and is involved in many cellular reactions such as the synthesis of purines, the metabolism of amino acids, the conversion of glycine to serum and the synthesis of lignin, chlorophyll and choline, as well as evapotranspiration cycle (Ibrahim *et al.* 2013). This compound can be used as a new economic organic compound and an organic fertilizer to improve the productivity of the plants and the preservation of their nutrients (Al-Qubaie 2012).

Humic acid is a natural antioxidant that has various biochemical effects in plants by increasing nutrient uptake, preserving vitamins and increasing amino acids in plant tissues. Humic acid is extensively used by farmers around the world owing to its numerous benefits. For example, it contributes to increased respiration, roots, stem growth, fresh and dry weights, P, K, Fe, Cu, Zn and Ca uptake by roots and the synthesis of plant enzymes and hormones. The higher the amount of humic acid is, the greater the antioxidant activity will be and the more resistant the plants will be to diseases and heat and chilling stresses (El-Bassiouny Hala *et al.* 2014; Syedabadi and Armin 2014).

To control pathogens and enhance soil safety, humic acid increases the uptake of nutrients by the plants, the availability of minerals and finally, the quality of the crop (Mauromicale *et al.* 2011). Humic acid can be supplied to the plants either by soil incorporation in liquid and powdered forms or by foliar application (Ulukan *et al.* 2008). The foliar application and soil incorporation of humic acid increase the amount of auxin, cytokinin and gibberellin hormones in plants (Abdel Mawgoud *et al.* 2007). The most mentioned biological benefits of humic acid in plants include better plant growth by increasing meiosis, more root growth, the increase in dry matter yield, the increase in grain production, the improvement of vitamins in plants, the increase in the plant membrane permeability and faster uptake of nutrients (Eyheraguibel *et al.*

2008). Humic acid affects plant root and shoot growth, but its greatest impact is on plant roots. It increases root volume by influencing the root system (Sabzevari *et al.* 2007).

Given the effective role of humic acid and folic acid in increasing plant yield and other traits and their role in enhancing the yield and quality of bulbous plants, the present study aimed to explore the effect of foliar application of these two acids on physiological, quantitative and quality traits of the tuberose.

Materials and Methods

The experiment was carried out as a field trial in the Faculty of Agriculture, Agriculture and Natural Resources University of Gorgan, Iran, based on a randomized complete block design with three replications. Two factors were used independently in this experiment. The first factor was the foliar application of growth stimulators (no application as the control, folic acid and humic acid at the rates of 50, 100 and150 mg L^{-1}), and the second factor was the foliar application time at 30, 50 and 70 days after planting (DAP). The tuberose bulbs were planted in late April of 2016. The uniform bulbs of tuberose cv. ‘Dezful’ with a diameter of 2 cm and relative weight of 45 g were received from the Tuberose Production Center of Dezful, Iran, and were planted in the field at the depth of 7 cm. During the first two weeks after planting, all plants were similarly irrigated and fed to make the plants ready for foliar application. After the growth of the leaves, the foliar application was performed at three steps according to the experimental design. Sigma and Merck brands of humic acid and folic acid, respectively, were used in this experiment. To increase the uptake efficiency of the nutrients, some drops of Twin as foliar soap (0.5%) were added to the solutions. The plants were sprayed in the early morning and they were irrigated the next day. Each tuberose plant was completely sprayed with the solution so that the whole surface of the plants was washed with the solution. The control plants were sprayed with distilled water and foliar soap. After five months, when the plants reached commercial maturity and a pair of lower florets opened up, the flowers were cut from the crown using secateurs and were sent to the laboratory for measurements. The recorded traits included spike length, stem length and diameter, shoot fresh weight, floret number, leaf number and area, flower emergence time, bulblet number, root development depth, N, P and K content, vase life and total chlorophyll content.

Measurement of internal pigments

Leaf chlorophyll was estimated by a non-destructive method with a SPAD-502 chlorophyll-meter. Chlorophyll-meters allow quickly estimating the chlorophyll content of the plant tissues without destroying them. This trait was determined by measuring the red light (650 nm) and infrared (950 nm) wavelengths on the leaves. Chlorophyll absorbs red light, but it transmits infrared light. The device uses the difference in light passing at these two wavelengths to provide the SPAD. It should be noted that SPAD does not specify chlorophyll content, rather, it is an estimation of chlorophyll concentration. SPAD has a strong correlation with leaf chlorophyll content (Hoel and Solhaug 1998).

Measurement of nitrogen (N), phosphorous (P) and potassium (K)

To measure the effect of spraying treatments on the uptake of some elements, leaf samples were collected from the middle of the plants before the flower harvest (in early August when the concentration of nutrients in plants almost reached a plateau). Then, they were washed with distilled water, were oven-dried at 65 °C for 48 hours and were ground. N content of the samples was measured by the ash-extraction method using an auto-Kjeldahl apparatus. The concentrations of other nutrients were determined by the dry ash-extraction method after preparing the extract. The P content of the extract was measured by the phosphor-vanadate method. The K content was estimated with a flame-photometer (Emami 1996).

Vase life was assessed by considering the color change of the flowers, floret shedding, the opening of the flowers and the viscosity of the stem end (Burchi *et al.* 2005). Leaf area was measured with a digital leaf area meter (the AT-Delta0-T model) (Dastyaran and Hosseini Farahi 2014).

Flowering stems and inflorescences were daily monitored to record the time of their emergence. Also, their length was measured with a ruler. A digital caliper was used to find out stem diameter. Finally, shoot fresh weight was measured with a digital FX-300 scale (0.001-g precision).

Data were analyzed using the SAS (ver. 9.1) software package and the means were compared by the LSD test.

Results and Discussion

The analysis of variance (ANOVA) revealed the significant effect of humic acid and folic acid treatments on the recorded physiological and morphological traits, except leaf number (Table 1).

Morphological traits

According to the results of ANOVA, the effects of foliar application of humic acid and folic acid treatments were significant on stem length, spike length, stem diameter, floret number, leaf area, bulblet number, root development depth and flower fresh weight (Tables 1). The comparison of means showed that as the rate of humic acid was increased, the morphological traits of the tuberose plants were improved and the lowest values were observed on the control plants (Table 2). Among the treated plants, the plants exposed to foliar application of humic acid at 150 mg L^{-1} exhibited the highest stem length, spike length, stem diameter and shoot fresh weight of 58.33 cm, 43.66 cm, 9.62 cm and 146.7 g, respectively (Table 2). Among the humic acid levels, the highest bulblet number and the deepest root system were related to the plants treated with 50 mg L^{-1} humic acid (Table 2). It can be drawn from Table 2 that there was a significant difference between some humic acid and folic acid treatments. Also, the results in Table 2 shows that the longest vase life (9.66 days) was associated with the application of 150 mg L^{-1} humic acid. Control flowers exhibited the shortest vase life of 6.66 days.

Table 1. Analysis of variance of the effect of folic acid and humic acid treatments on morphological and physiological traits and the nutrients uptake of tuberose.

Sources of variation	df	Stem length	Spike length	Stem diameter	Floret number	Leaf number	N (%)	P (%)	K (%)
Blocks	2	1.22^{ns}	8.65^{ns}	3.72**	7.76^{ns}	0.12^{ns}	0.004^{ns}	0.003^{ns}	0.132^{ns}
Treatments	6	83.46**	54.90**	1.16*	76.30**	0.98^{ns}	0.105**	0.195**	0.509**
Error	12	6.14	6.09	0.31	2.37	0.53	0.003	0.017	0.783
Coefficient of variation		4.952	6.582	6.35	3.65	10.21	2.513	14.214	15.565

ns: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$.

Table 1 continued.

Sources of variation	df	Leaf area	Bulblet number	Root development depth	Fresh weight	Chlorophyll	Flower emergence time	Vase life
Blocks	2	143.70^{ns}	6.95^{ns}	1.38^{ns}	71.29^{ns}	0.57^{ns}	304.76^{ns}	0.14^{ns}
Treatments	6	3839.37**	33.65**	17.51**	207.54**	59.28**	898.21^{ns}	4.75**
Error	12	258.52	3.03	1.13	19.06	2.43	484.21	0.20
Coefficient of variations		9.479	9.571	6.680	3.286	3.399	16.858	5.284

ns: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$.

Table 2. Means of the effect of folic acid and humic acid treatments on morphological and physiological traits and the nutrients uptake of tuberose.

Treatment	Stem length (cm)	Spike length (cm)	Stem diameter (mm)	Floret number	Leaf Area (cm^2)	Bulblet number	Root development depth (cm)
F50	47.83 cd	40.00 ab	8.87 ab	42.00 bc	185.55 bc	18.00 b	15.90 bc
F100	51.83 bc	40.33 ab	9.31 ab	47.00 a	188.77 b	18.00 b	19.50 a
F150	43.33 e	33.83 cd	8.73 ab	41.66 bc	228.00 a	17.33 bc	17.70 ab
H50	48.00 cd	36.16 bc	8.32 bc	40.00 c	149.71 d	25.33 a	16.95 bc
H100	55.00 ab	37.33 bc	8.78 ab	48.33 a	157.07 cd	17.33 bc	15.30 cd
H150	58.33 a	43.66 a	9.62 a	43.66 b	185.55 bc	16.66 bc	12.30 e
Control	46.00 e	31.00 d	7.72 c	35.00 d	131.27 e	14.66 c	13.80 de

Means with different letter(s) in each column are significantly ($p \leq 0.05$) different according to the LSD test.

Table 2 continued.

Treatment	Fresh weight (g)	Chlorophyll	Vase life (day)	N (%)	P (%)	K (%)
F50	133.3 b	42.16 d	7.33 bc	2.23 c	0.66 b	1.55 bc
F100	126.7 bc	42.26 d	8.00 b	2.40 b	0.84 b	1.97 b
F150	125.0 c	45.56 c	9.33 a	2.57 a	1.19 a	2.49 a
H50	130.0 bc	49.20 b	8.00 b	2.43 b	0.79 b	1.47 bc
H100	141.7 a	49.60 ab	9.66 a	2.66 a	1.15 a	1.90 b
H150	146.7 a	52.00 a	10.00 a	2.67 a	1.90 a	1.94 b
Control	126.7 bc	40.46 d	6.66 c	2.23 c	0.61 b	1.34c

Means with different letter(s) in each column are significantly ($p \leq 0.05$) different according to the LSD test.

There are various reports about the effect of humic acid. Humic acid has a direct impact as a semi-hormone compound and some indirect effects such as the increased uptake of nutrients via its chelating and reducing properties, the maintenance of membrane permeability, the increase in the metabolism of soil microorganisms and the enhancement of root and stem growth (Nardi 2002). The application of humic acid contributed to increasing root development depth and bulblet number. In a research program, the effect of humic acid was studied on the root growth of maize plants and the results showed that 3 mM humic acid at low and high concentrations of nitrate could improve the development of maize root system and increase the ratio of root fresh weight to its dry weight (Cordeiro *et al.* 2011). A study on the effect of humic acid on the growth of soybean, peanut and clover indicated that root weight and node number were increased in response to 400-800 mg humic acid (Tan and Tantiwiramanond 1983). The increase in bulblet number and root development depth can be related to the fact that humic acid enhances chlorophyll and so, the photosynthesis rate and dry matter production. In other words, it can be said that humic acid increases root growth by its semi-hormonal effects, thereby increasing the number of bulblets and root development depth (Jones *et al.* 2004; Ghasemi *et al.* 2012). In another study on the olives exposed to 0, 200 or 1000 mg humic acid in the fertilized pots, humic acid increased root/shoot ratio to a greater extent than root weight (Tattini *et al.* 1990). On the other hand, it has been established that because of its high cation exchange capacity, humic acid can increase the availability of beneficial nutrients and reduce toxic elements and heavy metals in the plant roots (Ghasemi *et al.* 2012).

In our study, humic acid improved stem and spike length as compared to the control. It has been found that humic acid can contribute to the growth increase of the main stem (Eyheraguibel *et al.* 2008). Stem length is reportedly a trait that is the most responsive to the humic acid application (Ulukan 2008). The accelerating effect of humic acid on stem growth can be associated with its impact on the activity of root H-ATPase and nitrate distribution across roots and stems, which in turn changes the distribution of cytokinins, polyamines and abscisic acid (Rubio *et al.* 2009).

Leaf area and chlorophyll content

According to the results of ANOVA (Table 1), the effect of foliar application of humic folic acid and folic acid treatments was significant on the total chlorophyll. The highest chlorophyll content (52 mg g^{-1}) was obtained from the application of 150 mg humic acid (Table 2). The leaf area index was also significantly influenced by the increase in the rates of humic acid and folic acid. The highest leaf area (228 cm^2) belonged to 150 mg folic acid and the lowest value (131.27 cm^2) was related to the control (Table 2). These findings are consistent with the study of Tailin *et al.* (2001). It seems that higher rates of folic acid and humic acid were beneficial for increasing biomass and photosynthesis pigments so that with the increase in leaf area index, more radiation was absorbed, resulting in higher photosynthesis and carbon fixation and better growth of the plants (Rios *et al.*

2009).

Humic acid increases plant yield through positive physiological impacts such as the effect on plant cell metabolism and the increase in leaf chlorophyll content (Nardi 2002). Foliar or soil application of humic acid increases the concentration of growth regulators, especially auxins, cytokinins and gibberellins in the plant tissues (Edwards and Fletcher 1998; Abdel Mawgoud 2007). Also, humic acid contributes to more longevity of leaves and higher leaf area by increasing intracellular metabolism and leaf chlorophyll content (Nardi 2002). High microbial activity in the organic matter enhances growth regulators, e.g. cytokinin, gibberellin and auxin, and thereby increases photosynthesis activity and carbohydrate synthesis (Krishnamoorthy and Vajranabhiah 1986).

Nutrients

Humic acid and folic acid treatments significantly influenced the N, P and K content of tuberose leaves (Table 1). Comparison of means (Table 4) showed that leaf N content was significantly increased with the increase in the rate of humic acid and folic acid application. The highest N and P content (2.67 and 1.90 percent) was observed in the plants treated with 150 mg L^{-1} humic acid. Among folic acid treatments, the highest N and P content (2.57 and 1.93 percent) was related to the 150 mg L^{-1} rate (Table 4). Plants treated with 150 mg L^{-1} folic acid showed the highest K content (2.49 percent), significantly differing from the control.

The correlation coefficients of leaf nutrients with the quality characteristics of tuberose are presented in Table 3. Leaf N content had a positive and significant correlation with the total chlorophyll (0.69), bulblet number (0.48) and post-harvest life (0.93) of tuberose. The concentration of P in the tuberose leaf samples was also significantly correlated with total chlorophyll (0.71), vase life (0.78) and bulblet number (0.47). Therefore, adequate N and P supply have a considerable effect on prolonging the post-harvest life of tuberose. Leaf K percentage, was not significantly correlated with the bulblet number and vase life of tuberose but was moderately correlated with the total chlorophyll (r = - 0.51) at $p \leq 0.05$ (Table 3).

Studies have shown that the application of humic materials enhances the uptake of nutrients and improves plant growth (Celik *et al.* 2012). The enhancement of morphological and physiological characteristics in plants fed by humic acid lies on its effect on improving the uptake of nutrients (N, Cu, Fe and Zn), chlorophyll content, and photosynthesis. According to Chen and Aviad (1990), humic acid increases plant yield by enhancing plant growth including roots, leaf area, plant biomass and tissue permeability and also by improving photosynthesis rate and nutrient uptake. It has been established that there is a significant linear relationship between Fe concentration and plant yield so that Fe application entails the increase in chlorophyll content, photosynthesis (Davarpanah *et al.* 2013) and vegetative growth (Amaliotis *et al.* 2002). In a study on the effect of humic compounds on improving Fe chelates efficiency in lemon trees, it was reported that humic compounds increased fruit weight (Sanchez-Sanchez *et al.* 2006). Fe deficiency stunts leaf

Table 3. Correlation coefficients among leaf nutrient contents and quality traits of tuberose in the field conditions.

	N	P	K	Total chlorophyll	Vase life	Bulblet number
N	1					
P	-0.73**	1				
K	-0.23ns	-0.02ns	1			
Total chlorophyll	0.69**	0.71**	-0.51*	1		
Vase life	0.93**	0.78**	-0.22ns	0.73**	1	
Bulblet number	0.48*	0.47*	0.32ns	0.24ns	0.46*	1

ns: not significant; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$.

growth and cell division and reduces the chlorophyll and cytochrome content (Marschner 1995). The increase in chlorophyll content by Fe application has been reported also in other plants (Mahmed *et al.* 2010). Fe is an important cofactor of many biochemical enzymes and although it is not present in the chlorophyll structure, it is necessary for chlorophyll synthesis (Marschner 1995). Also, the application of humic acid reduces chlorosis in plants which may be rooted in the capability of humic acid to maintain soil Fe in an absorbable and metabolizable form. This phenomenon can be effective in alkaline and calcareous soils, which are usually suffering from the deficiency of absorbable Fe and organic matter (Celik *et al.* 2011; Rahii *et al*. 2012). The increased concentration of Fe in leaves is the direct result of the foliar application of folic acid that can be readily absorbed by leaves.

Conclusion

Presently, the symptoms of the deficiency of micronutrients like Fe, Zn and B are prevailing due to unbalanced use of chemical fertilizers, high lime content, high pH and the deficiency of organic matter in soils. The concurrent use of chemicals and organic fertilizers is an approach emphasized by nutritionists in recent years. We found that the application of folic acid and humic acid improves flower marketable quality and morphological and physiological characteristics of tuberose through increasing nutrient efficiency. The foliar application of folic acid and humic acid supplied the plants with the nutrients directly and improved their uptake and mobilization. According to the results, it is recommended to foliar application of 150 mg L^{-1} humic acid or 150 mg L^{-1} folic acid on tuberose plants at the three specified stages to increase the yield and quality of tuberoses.

Conflict of Interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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اثر محلول‌پاشی اسید هیومیک و اسید فولیک بر بهبود ویژگی‌های بازارپسندی گل مریم (*Polianthes tuberosa*)

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چکیده

گل مریم از مهم‌ترین گل‌های شاخه بریده در ایران و جهان به شمار می‌رود.با توجه به این‌که تغذیه در رشد، نمو و کیفیت گیاهان زینتی از اهمیت زیادی برخوردار است،این مطالعه با هدف بررسی تاثیر سطوح مختلف اسید هیومیک و اسید فولیک بر عملکرد، صفات کمی و کیفی گل مریم (*Polianthes tuberosa* L.) در قالب طرح بلوک‌های کامل تصادفی با سه تکرار در سال ۱۳۹۵ در محوطه دانشکده کشاورزی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان انجام شد. اسید هیومیک و اسید فولیک در چهار سطح (صفر، ۵۰، ۱۰۰، ۱۵۰ میلی‌گرم در لیتر) مصرف گردید. محلول‌پاشی در سه نوبت ۳۰، ۵۰ و ۷۰ روز پس از کشت صورت گرفت. در این آزمایش، طول سنبله، طول و قطر ساقه، وزن‌تر اندام هوایی، تعداد گلچه، تعداد و سطح برگ، زمان ظهور گل، تعداد سوخک تولید شده، عمق توسعه ریشه، درصد نیتروژن، فسفر و پتاسیم، عمر گلجایی و کلروفیل کل اندازه‌گیری شد. نتایج تجزیه واریانس داده‌ها نشان داد که کاربرد اسید هیومیک باعث افزایش معنی‌دار صفات اندازه‌گیری شده،به جز تعداد برگ و زمان ظهور گل شد.با افزایش مصرف اسید هیومیک تا ۱۵۰ میلی‌گرم در لیتر میزان کلروفیل کل برگ، ۵۲ درصد افزایش یافت که با تیمار شاهد اختلاف معنی‌داری داشت.محلول‌پاشی اسید هیومیک و اسید فولیک باعث افزایش نیتروژن، فسفر و پتاسیم در برگ‌ها شد و بیشترین میزان تحت تاثیر غلظت ۱۵۰میلی‌گرم در لیتر به‌دست آمد. براساس نتایج این مطالعه، تیمار ۱۵۰ میلی‌گرم در لیتر اسید هیومیک و اسید فولیک بیشترین تاثیر را بر افزایش صفات کمی و کیفی گل مریم داشت.

واژه‌های کلیدی: اسید هیومیک؛ کلروفیل کل؛ گل مریم؛ نیتروژن؛ محلول‌پاشی