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THE USE OF HUMIC ACID AND EM IN REPLACEMENT OF NPK CHEMICAL FERTILIZATION FOR THE PRODUCTION OF *GARDENIA JASMINOIDES* POT PLANTS

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ABSTRACT: A pot experiment was conducted under greenhouse conditions at the Hort. Res. Inst., ARC, Giza, Egypt during the two successive seasons 2014 and 2015 to explore the possibility of using humic acid (H) and/or effective microorganisms (EM) instead of NPK chemical fertilization for producing *Gardenia jasminoides* pot plants.

Obtained results indicated that NPK fertilization treatment, as well as, humic acid and/or EM treatments caused great and considerable enhancement in different vegetative growth characters, flowering parameters and chemical constituents in comparison with control treatment. Hence, both NPK and other stimulant treatments (H and/or EM) gave nearly similar values for growth and flowering traits.

Therefore, it is advised, from the economical and environmental point of view, to use such stimulants (H and /or EM) in replacement of NPK chemical fertilizers for producing good quality pot plants of *Gardenia jasminoides*.

Key words: *Gardenia jasminoides*, humic acid, effective microorganisms (EM), NPK, vegetative growth, flowering, chemical constituents.

INTRODUCTION

Gardenia jasminoides, Ellis belongs to family Robiaceae is native to China. It is an evergreen shrub with glossy, bright green leaves and double white highly fragrant flowers, 5-10 cm across commonly borne singly in the leaf axes (Williamson, 1975). Gardenia grows successfully outdoors and in large containers and it does well indoors as pot plant. It requires a rich, moist acidic (pH 5-5.5) and well-drained soil high in organic matter. In addition, it has poor to low salt tolerance and moderate wind resistance (Kobayashi and Kaufman, 2006). In regard to landscape uses, Gardenia plants with their glossy, dark green foliage, make a great foundation in the landscape. It is effectively used as either focus or background in informal plantings and is a popular cut flower for the florist. Concerning growing Gardenia indoors, it should receive plenty of bright light, preferably direct sunshine for at least half a day in a sunny south or west window. It prefers temperature close to 16 ^{o}C at night and 21-24 ^{o}C during the day. Maintain uniform moisture and good drainage in the growth medium (Kobayashi and Kaufman, 2006).

The role of NPK in augmenting vegetative growth, flowering traits and chemical constituents but delaying flowering date was reported by many authors such as Badran *et al.* (1989 a) and Abdou (2002) on borage; Badran *et al.* (1989 b) on calla; Khalil and Helal (1998) on geranium; Badran *et al.* (2001), Sayed (2004) and

Hassan (2016) on Gladiolus. The positive effect of NPK fertilization was also demonstrated by Ashour (2006) on *Ficus elastica*; Barsoom (2008) on *Voila adorata*; Kandeel *et al.* (2008) on *Jasminum grandiflorum*; El- Sayed (2012) on Seashore paspalum and Badran *et al.* (2016) on *Moringa oleifera*. Meanwhile, the role of humic acid in promoting growth, flowering and/or chemical constituents was obtained on *Ficus benjamina* (Siraj *et al.*, 2001); four ornamental plants (Evans and Li, 2003); pot marigold (Azzaz *et al.*, 2007); turfgrass plants (El-Sayed *et al.*, 2008 and El- Sayed. 2012) *Dracaena* and *Ruscus* (Abd-Elfattah *et al.*, 2009); *Gladiolus* (Ahmed *et al.*, 2013) and *Gazania* (Yukun *et al.*, 2014). The stimulating influence of EM was revealed by Thach *et al.* (1999) on orchid plants, Abd-Elraoof (2009) on borage; Romualad and Tomasz (2010) on Gerbera; Ashour (2010) on jojoba; El-Sayed (2012) on Seashore paspalum; Ali (2013) on pot marigold; Abd-Ellatif (2014) on Lavander; Wang *et al.* (2015) on three container plants; Schroeter–Zekrzewska *et al.* (2016) on geranium and Hassan (2016) on *Gladiolus*.

The aim of the present investigation was to explore the possibility of using some natural stimulants, like humic acid and effective microorganisms (EM), in replacement of NPK mineral fertilization in producing quality pot plants of *Gardenia jasminoides.*

MATERIALS AND METHODS

A pot experiment was conducted in the greenhouse of the Dept. of Floriculture, Hort. Res. Institute, Agric. Res. Center, Giza, Egypt during 2014 and 2015 successive seasons to explore the influence of five fertilization treatments on growth, flowering and chemical constituents of *Gardenia jasminoides*, Ellis plants.

Rooted cuttings of Gardenia with average height of 5 cm, obtained from Safwat Habib nursery, Kerdasa, Giza, were transplanted on the first week of March for both seasons to 20 cm plastic pots filled with peatmoss. After transplanting, Mooncut fungicide was added as soil drench, and chelated ferrous at the rate of 150 ppm was foliar sprayed every month along the whole growing season. The experimental layout of this trial was complete randomized block design with three replicates and five plants/replicate. The five fertilization treatments were control, NPK (Kristalon 19:19:19), humic acid (H), effective microorganisms EM) and H + EM. NPK (Kristalon) was added at the rate of 2.0 g/pot every 2 weeks, humic acid (H) and EM were supplied at the rate of 5 cm^3/l and 1 cm^3/l, respectively, every 2 weeks. The three fertilizers were applied as soil drench after one week from transplanting for the two seasons. Physical and chemical properties of the used peatmoss, for both seasons are given in Table (a).

Table a. Physical and chemical properties of the used peatmoss in the two seasons.

Properties	Values	Properties	Values
Org. Matter (%)	90-95	**P (%)**	0.23
Ash (%)	8-10	**K (%)**	1.77
Density (Vol D.W. mg/l)	80-90	**Fe (ppm)**	421.0
pH	3.4	**Mn (ppm)**	27.0
Water retention cap. (%)	60-75	**Zn (ppm)**	41.0
Salinity (mg/l)	0.3	**Cu (ppm)**	8.8
N (%)	1.09	**Mg (ppm)**	3.3

On the first week of Oct. for each season the following vegetative growth characters and flowering parameters were recorded: plant height (cm), stem diameter (mm), branch number/plant, leaf area (cm^2), leaf number/plant, leaves fresh weight/plant, flowering date (day), flowers number/plant and flowers fresh weight/plant (g.).

Chemical constituent determinations:

The three photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids contents) were colourmetrically determined according to Saric *et al.* (1976). While, nitrogen, phosphorus and potassium % were determined by the modified microkjeldahl method (Plummer, 1971) for nitrogen; colormetrically (Jackson, 1958) for phosphorus and flame- photometer (Piper, 1950) for potassium. Meanwhile, total carbohydrates were estimated following the method of Dubois *et al*. (1956), while each of total indoles and total phenols were determined following the method described by Daniel and George (1972).

Obtained data for vegetative, flowering and chemical constituent parameters were statistically analyzed according to the L.S.D. method described by Little and Hills (1978).

RESULTS AND DISCUSSION

Vegetative growth characters:

Obtained data in Tables (1 and 2) showed that all six vegetative growth characters of *Gardenia jasminoides* plants, namely, plant height, stem diameter, branch number/plant, leaf area, leaves number/plant and leaves fresh weight/plant were significantly increased, in the two seasons, in comparison with those of control plants. However, the highest values for stem diameter, branch number, leaves number and leaves fresh, weight/plant were given due to NPK and humic + EM treatments, while, the best results for plant height and leaf area were obtained from humic acid and/or EM treatments. These results were almost identical in both seasons, (Tables 1 and 2). Therefore, it could be said that the humic acid + EM combined treatment was the most overall effective one in producing the tallest plants, thickest stems, most branch and leaves number and leaf area and heaviest leaves fresh weight. The increase in plant height, stem diameter, branch number, leaf area, number of leaves and leaves fresh weight due to such treatment (H+ EM) reached 48.5, 22.5, 48.8, 31.7, 34.1 and 14.1% in comparison with control treatment in the first season. Similar trend was observed in the second season as clearly shown in Tables (1 and 2).

Table 1. Effect of fertilization treatments on plant height, stem diameter and branch number of *Gardinia jasminoides* during 2014 and 2015 seasons.

Treatments	Plant height (cm)		Stem diameter (mm)		Branch number/plant	
	2014	2015	2014	2015	2014	2015
Control	32.8	30.6	4.62	4.25	3.28	3.51
NPK	36.6	34.0	5.82	5.62	4.94	4.72
Humic acid (H)	48.1	44.2	5.11	4.92	4.36	4.25
EM	49.3	43.8	5.08	4.88	4.44	4.16
H + EM	48.7	43.0	5.66	5.58	4.88	4.54
L.S.D. 5%	2.4	2.7	0.36	0.40	0.42	0.35

EM= Effective microorganisms.

Table 2. Effect of fertilization treatments on leaf area, leaves number and leaves fresh weight of *Gardinia jasminoides* during 2014 and 2015 seasons.

Treatments	Leaf area (cm^2)		Leaves number/plant		Leaves F.W. (g)	
	2014	2015	2014	2015	2014	2015
Control	18.9	16.0	53.3	51.8	19.9	17.8
NPK	20.6	17.8	70.2	66.6	24.7	22.5
Humic acid (H)	24.8	21.6	64.5	61.9	21.5	19.3
EM	24.6	22.3	64.9	62.3	21.4	20.5
H + EM	24.9	22.1	71.5	65.8	23.7	22.7
L.S.D. 5%	1.4	1.6	4.4	5.6	1.2	1.1

EM= Effective microorganisms.

The role of NPK in augmenting various vegetative growth characters was revealed by Badran *et al.* (1989 a) on borage; Badran *et al.* (1989 b) on calla, Badran *et al.* (2001) on Gladiolus; Ashour (2006) on *Ficus elastica*; Barsoom (2008) on *Viola odorata* Kandeel *et al.* (2008) on *Jasminum grandiflorum*; El-Sayed (2012) on Seashore Paspalum and Badran *et al.* (2016) on *Moringa oleifera*. While, that of humic acid was reported by Siraj *et al.* (2001) on *Ficus benjamina*; Azzaz *et al.* (2007) on pot marigold; El-Sayed (2008) and El- Sayed *et al.* (2012) on turfgrasses; Abd-Elfattah *et al.* (2009) on *Dracaena* and *Ruscus* and Yukun *et al.* (2014) on *Gazania*. Meanwhile, other authors emphasized the importance of EM in enhancing vegetative growth of orchid (Thach *et al.,* 1999); borage (Abd-Elraoof, 2009); Jojoba (Ashour, 2010); Seashore Paspalum (El-Sayed. 2012); pot marigold (Ali, 2013); Lavander (Abd-Ellatif, 2014); three container plants (Wang *et al.,* 2015) and Gladiolus (Hassan, 2016).

Flowering parameters:

Flowering date was differently and significantly influenced by the examined fertilization treatments in the present investigation as NPK treatment caused significant delay (9.8 and 12.6 days in the first and second seasons, respectively) in regard to unfertilized treatment, (Table 3). In contrast, humic acid, EM and humic + EM treatments produced significantly earlier flowers than those of unfertilized plants (about 8-10 days in the first season and 8-9 days in the second season) as illustrated in Table (3). Concerning number of flowers/plant and flowers fresh weight/plant, both flowering parameters were dramatically increased , in the two seasons, due to all four tested treatments comparing to control treatment. Both number and fresh weight of flowers, for each one of the four fertilized treatments were almost gave as much as double values of those recorded for control plants. However, NPK and humic + EM treatments were superior to humic or EM treatments as clearly indicated in Table (3).

In accordance with these findings concerning NPK were those found by Badran *et al.* (1989a) and Abdou (2001) on borage; Badran *et al.* (1989b) on calla; Badran *et al.* (2010) and Hassan (2016) on gladiolus; Barsoom (2008) on *Viola odorata* and Kandeel *et al.* (2008) on *Jasminum grandioflorum*. Meanwhile, the efficient role of humic acid on flowering aspects was mentioned by Evans and Li (2003) on four ornamental plants; Azzaz *et al.* (2007) on pot marigold; Ahmed *et al.* (2013) on gladiolus and Yukun *et al.* (2014) on Gazania. While that of EM was pointed out by Thach *et al.* (1999) on orchid; Abd-Elraoof (2009) on borage; Romuald and Tomasz (2010) on Gerbera; Ali (2013) on pot marigold; Schroeter–Zekrzewska *et al.* (2016) on geranium and Hassan (2016) on Gladiolus.

Table 3. Effect of fertilization treatments on flowering date, flower number and flower fresh weight of *Gardinia jasminoides* during 2014 and 2015 seasons.

Treatments	Flowering date (day)		Flowers number/plant		Flowers F.W. (g)	
	2014	2015	2014	2015	2014	2015
Control	50.5	44.4	3.61	4.02	10.3	11.7
NPK	60.3	57.0	8.35	8.40	39.3	43.3
Humic acid (H)	40.8	36.1	7.80	7.74	32.9	33.1
EM	42.2	35.8	7.62	7.66	31.9	32.9
H + EM	40.1	35.3	8.20	8.18	38.3	38.8
L.S.D. 5%	4.7	5.1	0.49	0.72	2.8	2.6

EM= Effective microorganisms.

Chemical constituents:

Photosynthetic pigments:

Table (4) showed that the contents of the three photosynthetic pigments, chlorophyll a, chlorophyll b and carotenoids in the leaves of Gardenia plants were significantly promoted due to NPK, humic acid, EM and humic + EM treatments in comparison with those of control treatment in both first and second seasons. Among such four treatments, NPK gave significantly higher values, for the three pigments in both seasons, than those recorded by humic and/or EM treatments as illustrated in Table (4). In agreement with these results concerning NPK were those of Badran *et al.* (2001), Sayed (2004) and Hassan (2016) on Gladiolus; Ashour (2006) on *Ficus elastica*; Barsoom (2008) on *Viola odorata*; Kandeel *et al.* (2008) on *Jasminum*; El-Sayed (2012) on Seashore Paspalum and Badran *et al.* (2016) on *Moringa*. Regarding humic acid were the findings of El-Sayed *et al.* (2008) on tifway sod; Abd-Elfattah *et al.* (2009) on *Dracaena* and *Ruscus* and El-Sayed (2012) on Seashore Paspalum. In relation with EM were the results given by Thach *et al.* (1999), Abd-Elraoof (2009), Ali (2013), Abd-Ellatif (2014), Schroeter–Zekrzewska *et al.* (2016) and Hassan (2016) on orchid, borage, pot marigold, lavander, geranium and *Gladiolus*, respectively.

Leaves percent of N, P and K:

Leaves percent of nitrogen, phosphorus and potassium were greatly and significantly stimulated as a result of supplying Gardenia plants with NPK, humic acid, EM and humic + EM treatments over the control plants as illustrated in Table (5). Among the pre-mentioned four treatments, NPK gave significantly higher N, P and K % values than the other three treatments. Moreover, such three treatments did not show significant differences. All above mentioned results proved to be true in the two seasons as shown in Table (5). The role of NPK fertilization in promoting nitrogen, phosphorus and potassium % was revealed by Badran *et al.* (1989b) on calla; Badran *et al.* (2001) and Sayed (2004) on *Gladiolus*; Barsoom (2008) on *Viola odorata*; Kandeel *et al.* (2008) on *Jasminum grandiflorum* and Badran *et al.* (2016) on *Moringa oleifera*. While that of humic acid was reported by Siraj *et al.* (2001). El- Sayed *et al.* (2008); Abd-Elfattah *et al.* (2009) and El-Sayed (2012) on *Ficus benjamina*, tifway sod, *Dracaena* and Seashore Paspalum, respectively. Similar response of N, P and K % to EM application was mentioned by Abd-Elraoof (2009), Ashour (2010), Ali (2013), Abd-Ellatif (2014) and Hassan (2016) on borage, jojoba, pot marigold, lavander and Gladiolus, respectively.

Total carbohydrates %:

Table (6) showed that total carbohydrates % was significantly increased in both seasons, due to the four tested fertilization treatments (NPK, humic acid, EM and humic + EM) over control treatment. However, NPK treatment was much more effective than the other three treatments as it increased carbohydrates % by nearly 100 % over control treatment, while the other three treatments increased it by more than 50%. These results proved to be true in the two experimental seasons, (Table 6). In close agreement with these results concerning NPK were the findings of Khalil and Helal (1998) on geranium, Sayed (2004) and Hassan (2016) on *Gladiolus*; Kandeel *et al.* (2008) on *Jasminum grandifloruum* and El-Sayed (2012) on Seashore paspalum. And regarding humic acid those found by El- Sayed *et al.* (2009) on tifway sod, Abd-Elfattah *et al.* (2009) on *Dracaena* and *Ruscus* and El-Sayed (2012) on Seashore Paspalum. Accordingly, EM was found to promote carbohydrates % as reported by El-Sayed (2012) and Hassan (2016) on Seashore Paspalum and *Gladiolus*, respectively.

Total indoles and total phenols:

Both total indoles and total phenols were significantly promoted, in both seasons, due to supplying Gardenia plants with NPK,

Table 4. Effect of fertilization treatments on chlorophyll a, chlorophyll b an carotenoids contents of of *Gardinia jasminoides* during 2014 and 2015 seasons.

Treatments	Ch. a (mg/g F.W.)		Ch. b (mg/g F.W.)		Carot. (mg/g F.W.)	
	2014	2015	2014	2015	2014	2015
Control	0.961	1.018	0.335	0.351	0.431	0.460
NPK	1.462	1.434	0.573	0.587	0.666	0.711
Humic acid (H)	1.280	1.350	0.420	0.549	0.552	0.610
EM	1.278	1.323	0.398	0.465	0.538	0.588
H + EM	1.340	1.329	0.440	0.532	0.575	0.632
L.S.D. 5%	0.063	0.071	0.025	0.032	0.034	0.044

EM= Effective microorganisms.

Table 5. Effect of fertilization treatments on the leaves % of N, P and K of *Gardinia jasminoides* during 2014 and 2015 seasons.

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	2014	2015	2014	2015	2014	2015
Control	2.23	1.96	0.270	0.252	1.38	1.31
NPK	3.04	2.66	0.463	0.409	1.76	1.63
Humic acid (H)	2.54	2.34	0.392	0.340	1.50	1.48
EM	2.48	2.30	0.400	0.325	1.52	1.52
H + EM	2.57	2.42	0.389	0.337	1.54	1.54
L.S.D. 5%	0.12	0.14	0.015	0.020	0.07	0.08

EM= Effective microorganisms.

Table 6. Effect of fertilization treatments on carbohydrates %, total indoles and total phenols of *Gardinia jasminoides* during 2014 and 2015 seasons.

Treatments	Total carbohyd. (%)		Indoles (mg/100 g)		Phenols (mg/100 g)	
	2014	2015	2014	2015	2014	2015
Control	20.5	15.2	0.442	0.302	0.111	0.094
NPK	40.8	38.0	0.588	0.479	0.322	0.296
Humic acid (H)	31.3	31.1	0.489	0.361	0.191	0.178
EM	31.7	32.2	0.492	0.358	0.179	0.172
H + EM	33.3	33.0	0.510	0.363	0.216	0.182
L.S.D. 5%	1.9	1.5	0.018	0.022	0.009	0.014

EM= Effective microorganisms.

humic acid, EM or humic + EM fertilization treatments in comparison with those of control plants. Among the four mentioned treatments, NPK was superior significantly over the other three treatment. Moreover, the combined humic + EM treatment gave higher values of indoles and phenols than each one alone. These results were almost identical for both first and second seasons as indicated in Table (6). In agreement with these results, regarding NPK were those of Ashour (2006) on *Ficus elastica* and El-Sayed (2012) on Seashore Paspalum, concerning humic acid those of El-Sayed (2012) on Seashore Paspalum and regarding EM were the findings of Ashour (2010), El-Sayed (2012) and Abd-Ellatif (2014) on jojoba, Seashore Paspalum and lavander, respectively.

In order to explain the beneficial roles of NPK fertilization in enhancing growth, flowering and chemical constituents of

Gardenia jasminoides, Ellis plants, it is convenient to refer , in brief, to the physiological roles of these elements, which considered to be the most common macronutrients in plant growth and development. Nitrogen is a constituent of all proteins, many enzymes and energy transfer materials such as chlorophyll, ADP and ATP. Growing plants must have nitrogen to form new cells and the rate of growth then becomes very nearly proportional to the rate at which nitrogen is supplied. Also, photosynthesis can produce soluble sugars from CO_2 and H_2O, but the process cannot go on to the production of protein in absence of nitrogen. Thus, a severe shortage of nitrogen will halt the process of growth and reproduction (Bidwell, 1974). Phosphorus, which has been called the key to life, is essential for cell division and the development of meristimatic tissue and it is very important for carbohydrate transformation due to multitude of phosphorylation reaction and to energy rich phosphate bond (Lambers *et al.,* 2000). Phosphorus compounds are essential for photosynthesis, the interconvention of carbohydrates and related glycolysis, amino acid metabolism, fat metabolism and biological oxidation. Lack of phosphorus, therefore, hampers metabolic processes such as the conversion of sugar into starch and cellulose (Devlin, 1972). Potassium is important for growth and elongation probably due to its function as an osmoticum and may react synergistically with IAA. Moreover, it promotes CO_2 assimilation and translocation of carbohydrates from the leaves to the storage tissues (Mengel and Kirkby, 1987).

Humic acid, nowadays, is widely used for production of most crops, namely, vegetables, fruit trees, field crops, medicinal and aromatic plants, flowers and ornamental plants, as well as, indoor container plants. Humic acid provides soil microbs with energy, increases the availability of nutrients in the soil through its influence on soil microbial activity, release more nutrients necessary for healthy growth, decrease phosphate fixing capacity of the soil, improves nutrients retention in the soil, enhances water holding capacity, improves soil structure and acts as a source of N, P and S for plants (Heng, 1989; Higa and Wididana, 1991 and Dorer and Peacock, 1997).

A commercial Japanese product, effective microorganisms (EM) is a biostimulant that contains more than 60 selected strains of effective microorganisms, viz, photosynthetic bacteria, lactic acid bacteria, yeast, actionomytes and various fungi that improves growth and health of plants (Primavesi, 1999). It have been introduced to the organic farming system in many countries, including Egypt. Treatment with EM led to larger stems. Darker green leaves and accelerated flowering. It is also effective in creating humus and improving soil properties, increasing plant disease resistance and may play a role in enhancing the enzymatic system in the plant tissues and consequently enhancing growth (Thach *et al.,* 1999 and Janas, 2009). Furthermore, Primavesi (1999) suggested that EM promotes stroma lamella formation, chlorophyll appearance during normal leaf growth and stimulates total sugars, as well as, total indoles and total phenols.

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استعمال حامض الهيوميك والكائنات الدقيقة الفعالة (EM) بدلا من التسميد الكيماوى (NPK) فى انتاج نباتات أصص الجاردينيا

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أجريت هذه التجربة تحت ظروف الصوبة الزجاجية بمعهد بحوث البساتين، مركز البحوث الزراعية بالجيزة، مصر خلال موسمى ٢٠١٤ ، ٢٠١٥ للتوصل لمعرفة امكانية استعمال كل من حامض الهيوميك والكائنات الدقيقة الفعالة (EM) كبديل للتسميد الكيماوى (NPK) فى انتاج نباتات الجاردينيا كنباتات اصص.

أظهرت النتائج أن كل من التسميد الكيماوى (NPK) وكذلك أى من حامض الهيوميك أو ال(EM) او كلاهما قد سببت زيادة وتحسنا واضحا فى الصفات الخضرية والزهرية والمكونات الكيماوية مقارنة بمعاملة الكنترول. وكان من الواضح أن كلا من التسميد الكيماوى والمنشطات الاخرى (حامض الهيوميك أو (EM) أو كلاهما معا) قد أعطت نتائج متقاربة لكل من الصفات الخضرية والزهرية وعلى ذلك فيمكن من وجهة النظر الاقتصادية والبيئية استعمال تلك المواد المنشطة (حامض الهيوميك و(EM) كبديل للتسميد الكيماوى فى انتاج نباتات أصص ذات صفات جيدة من نباتات الجاردينيا.