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Improving Vegetative Growth, Root Traits and some Chemical Constituents of *Duranta erecta* Var. *variegata* by Using Humic Acid, Salicylic Acid and Chitosan

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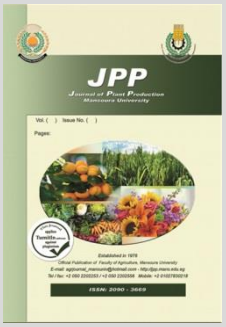

ABSTRACT

This experiment was conducted at greenhouse of Horticulture Research Station, Mansoura, Egypt during the two consecutive seasons of 2017 and 2018. The aim of the present work was to improve plant growth, roots traits and chemical constitutes of the sky flower(*Duranta erecta* var. *variegata*) by using humic acid (HA) at the rates of 2,4 and 6 ml/l and salicylic acid (SA) at the rates of 100,150 and 200 mg/l as well as chitosan (CH) at the rates of 100,200 and 300 mg/l.The obtained results revealed that HA, SA and CH applications at any rate significantly improved plant growth characters (plant height, number of branches per plant, fresh and dry weights per plant and leaf area) and root traits (root length and fresh and dry weights of roots per plant) when compared with control. In same time, all application treatments under study significantly increased total chlorophyll content, total nitrogen, phosphorus and potassium percentages in the leaves in comparison with control treatment (untreated plants). The best results in this connection (plant growth, root traits and chemical constituents) were recorded from 300 and 200 mg chitosan/l, followed by 6 ml humic acid/l then 200 mg salicylic acid/l during both seasons.

Keywords: *Duranta erecta* var. *variegata*, Humic and Salicylic Acids, Chitosan, Growth, Root and Chlorophyll.

INTRODUCTION

Sky flower (*Duranta erecta* var. *variegata*, L.) is a sprawling shrub or a short tree belongs to Verbenaceae Family. Duranta shrubs are evergreen and tolerate cut and shaping, so they are widely used in Egypt as a green fence in private and public gardens. West India is the original habitat of this plant. It can grow to 6 m height and can dispersal to an amounting to its width. It is widely planted as much for its striking creamy white and green variegatad foliage and for its lavender flowers that evidence roughly all year round (Huxley *et al*., 1992). It can be utilized as a hedge and bringing the potted garden, singular burst of form and color. Also it can be used as basket plant and a mounding bush as well as train into a standard (Rowezak, 2001). Sky flower can propagate by either seeds or stem cuttings (Robbins and Evans, 2006).

Organic fertilizers considered as one of the modern procedures to improve plant development (Ebrahimi and Miri, 2016). Due to the organic acids advantages to both plant and soil, it has been more and more utilized in arable and naturalist lands. Organic acids could enhance plant growth and productivity as well as soil chemical, physical and biological properties. Humic acid (HA), as one of these compounds that derived from humus and other natural resources, shows no harmful environmental influence on the perfection of the performance and can notably and effectually be utilized in changing the environment (Bulentasik *et al*., 2009 and Çelik *et al*., 2010). This acid can have favorable impacts on plant growth and increases the absorption of nitrogen, magnesium, phosphorus, calcium, and potassium by the plant (Sabzevari *et al*., 2009).

Exogenous applications of growth regulators showed to be effective in improving plant growth and chemical constituents (Hashmi *et al*., 2012 and Abdelkader and Hamad, 2014). Among these regulators, salicylic acid is an endogenous growth regulator, naturally found in plants in very small quantities. It consider as a chemical messenger that plays an important role in biotic and abiotic stress tolerance. Moreover, Gad *et al*. (2016) pointed out that, concerning SA, the lowest concentration (100 ppm) created the highest plants while 200 ppm increased leaf area (LA) . Number of leaves per plant and plant pigments (chlorophyll a, b and a+b as well as carotenoids) increased at 300ppm. Spraying *Ixora coccinea* plants with 400ppm enhanced fresh and dry weights of shoots and roots.

Chitosan came from chitin, which is considered as the second most abundant naturally materializing polysaccharides next to cellulose set in the plantlet (Rinaudo, 2006). The stimulatory effect of chitosan on plant growth may be attributed to an increase in availability and uptake of water and essential nutrients and increase enzyme activities (Guan *et al*., 2009). In addition, Amiri *et al*. (2016) indicated that chitosan application significantly increased root and shoot dry weights, leaf area index and chlorophyll content in maize and bean crops compared to control.

The main goal of this work was to improve plant growth, root traits and chemical constituents of *Duranta*

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erecta var. *variegata* by using humic acid, salicylic acid and chitosan under Dakahlia Governorate conditions.

MATERIALS AND METHODS

This study was conducted at green house of Horticulture Research Station, Mansoura, Egypt, during two consecutive seasons of 2017 and 2018.The objective of thr present work was to investigate the effect of humic acid(HA) at the rates of 2, 4 or 6 ml/l, salcylic acid(SA) at the rates of 100, 150 or 300 mg/l and chitosan at the rates of 100, 200 or 300 ppm on plant growth, root traits and chemical constituents of duranta erecta var. variegate plants. The layout of experiment was complete randomized block design with three replicates (each replicate contained 10 plants). Therefore, the study included 9 treatments + control treatment (without any applications). Duranta cuttings (15 cm. long) were dipped in IBA solution at 250 ppm for 5 minutes and then planted in plastic pots of 8 cm size on 20 March 2017 and 23 March 2018. After two months on 20 May 2017 and 23 May 2018, a mixture of sand and silt by 1:1 (v: v) with supplement calcium mono-phosphate ($CaH_4P_2O_8$) was prepared and packed in pots of 25 cm size and the soil was sterilized with fungal disinfectant No-Blight and seedlings were planted in the pots and were daily irrigated at the 1st week. Thereafter, duranta plants were irrigated every 3 days through the summer months.

Humic acid was added as a drench around the duranta plants, whereas salicylic and chitosan were used as a foliar application and the plants were sprayed by a hand sprayer until run off point. The bio-stimulants were done at 20 July 2017 and 23 July 2018 six timesat at fortnightly intervals during the growing season under greenhouse conditions (25±1ºC and 40-50% R.H.). All recommended agricultural practices of growing duranta plants were done when ever needed.

Data Recorded

1- Plant growth: Plant height (cm), number of branches / plant, fresh and dry weight g/plant and Leaf area (cm^2) were estimated after 15 days from the last application by taking 3 random guarded plants from each experimental unit. The leaf area calculated using the following equation then the average calculated for one leaf.

$$\text{Leaf area } (cm^2) = \frac{\text{leaf weights} \times \text{Square areas}}{\text{Square weights}}$$

2- Root traits: Root length (cm) as well as fresh and dry weight of roots/(g/plant) were determined. At the end of the experiment, duranta plants were removed from plastic pots. The culture mixtures surrounding the roots were removed by washing the roots system in running water over a sieve to ensure a minimum loss of the fine rootlets from the roots system.

3- Chemical constituents: a sample of dry leaves was randomly taken from each treatment for chemical analysis. Total nitrogen (%) was determined in dry leaves according to the methods described by Chapman and Pratt (1978). Total phosphorus percentage was determined in duranta leaves according to the methods adapted by Hucker and Catroux (1980). Potassium percentage was determined in sepals by using flame photometer according to the method described by Jackson (1973). Total chlorophyll (a +b) contents (mg/g fresh weight) were determined in fresh leaves of duranta plant according to the method outlined by Cherry (1973).

Statistical analysis:

Data were analyzed statistically using analysis of variance (ANOVA) and the mean differences were adjusted with Duncan's multiple range test at a 0.05 level of significance (**Steel, 1980**) using the statistical computer package program COSTAT.

RESULTS AND DISCUSSION

Plant growth:

As shown in Table (1) and illustrated in Fig. (1) that, all treatments of the bio-stimulants under study significantly increased the plant height, number of branches per plant as well as plant fresh and dry weights per plant and leaf area as compared with the untreated control. The highest values of duranta growth parameters were achieved with the treatments of chitosan at the rates of 300 and 200 mg/l, humic acid at the rate of 6ml/l then salicylic acid at the rate of 200 mg/l in the two seasons.

Table 1. Effect of humic acid (HA), salicylic acid (SA) and chitosan (Ch) on plant height (cm), number of branches per plant as well as fresh and dry weights (g) of *Duranta erecta* var. *variegata* plant during 2017 and 2018 seasons

Treatments	Plant height (cm)		Number of branches/plant		Fresh weight/plant (g)		Dry weight/plant (g)	
	2017 season	2018 season	2017 season	2018 season	2017 season	2018 season	2017 season	2018 season
Control	42.10i	44.82h	4.00f	4.67f	20.41i	21.95i	3.93f	4.22f
2 ml/l HA	48.07g	50.77f	6.33e	7.67de	24.38g	26.22g	5.07e	5.46e
4 ml/l HA	53.97e	57.64d	8.33cd	9.00cd	29.26e	31.25e	6.03d	6.44d
6 ml/l HA	61.73b	65.36b	10.00b	10.33bc	36.47b	39.00b	7.40c	7.92c
100 mg/l SA	45.23h	47.87g	6.00e	7.00e	22.24h	23.60h	4.29f	4.55f
150 mg/l SA	50.80f	53.92e	7.33de	8.67cde	27.43f	29.29f	5.30e	5.66e
200 mg/l SA	58.23c	62.20c	9.67bc	11.00b	34.40c	37.04c	7.04c	7.58c
100 mg/l Ch	55.23d	58.25d	8.00d	9.00cd	31.41d	33.72d	6.43d	6.91d
200 mg/l Ch	62.63b	66.30b	10.67ab	11.67ab	38.91a	41.42a	8.06b	8.58b
300 mg/l Ch	65.50a	70.17a	12.00a	13.00a	39.61a	42.43a	8.63a	9.25a

Means in the same column followed by the same letters are not significantly different according to Duncan's multiple test at ($p \leq 0.05$).

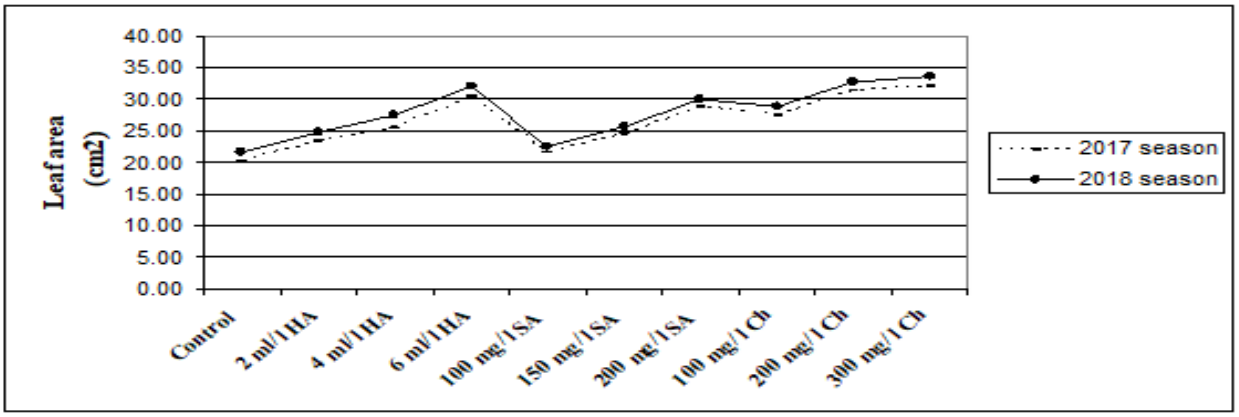

Fig. 1. Effect of humic acid (HA), salicylic acid (SA) and chitosan (Ch) on leaf area (cm^2) of *Duranta erecta* var. *variegata* plant during 2017 and 2018 seasons

The increases in plant height (cm) were about 55.58 and 56.56 % for chitosan at 300 mg/l, 46.63 and 45.83 % for humic acid at 6 ml/l, 38.31 and 38.78 % for salicylic acid at 200 ml/l over the control in 1st and 2nd seasons, respectively.

Also, the increases in fresh weight per plant were about 90.64 and 88.70 % for chitosan at 200 mg/l, 78.69 and 77.68 % for humic acid at 6 ml/l, 68.54 and 68.75 % for salicylic acid at 200 ml/l over the control in 1st and 2nd seasons, respectively (Table 1). In addition, the increases in leaf area were about 57.42 and 55.44 % for chitosan at 300 mg/l, 50.05 and 47.74 % for humic acid at 6 ml/l, 41.45 and 38.70 % for salicylic acid at 200 ml/l over the control in 1st and 2nd seasons, respectively (Fig. 1).

However, the stimulating influence of chitosan on plant growth may attribute to promote in the availability and uptake of water and major nutrients and improve enzyme activities (Guan *et al*., 2009). Moreover, Ibrahim (2010) on geranium plants and El-Kenawy (2017) on Thompson seedless grapevines plants, also have reported similar results. There is a correlation between the beneficial effect of SA on the synthesis of secondary metabolites with advance in growth, photosynthesis and nutrient content (Khanam and Mohammad, 2018). These results also found by Es-sbihi *et al.* (2020) who demonstrated that SA spraying on sage plants significantly increased plant growth. Furthermore, The positive influences of humic acid on cell membrane functions by elevating nutrient uptake, respiration, biosynthesis of ion absorption, nucleic acid, enzyme in order to they are hormone-like materials (Yang *et al*., 2004). HA used for plant nutrition, enhances development and plant growth due to its action on physiological and metabolic procedures (Eyheraguibel *et al*., 2008). Moreover, Said-Al Ahl *et al*. (2016) indicated that spraying by HA recorded the best results of plant height and number of branches compared to control.

Root traits:

Data of both seasons in Table (2) indicated that, all treatments under study (except the low rate of salicylic acid regarding the average fresh weight of roots) significantly increased root parameters as compared with the control (untreated plants). Concerning the impact of humic acid, salicylic acid and chitosan on fresh and dry weights of root per plant, the results cleared that, the significantly heaviest weights were obtained with the treatments of chitosan at the rates of 300 and 200mg/l, humic acid at 6ml/l and salicylic acid at 200mg/l, respectively. Furthermore, the increases in dry weight of roots per plant were about 96.51 and 97.54 % for chitosan at 300 mg/l, 86.03 and 84.84 % for humic acid at 6 ml/l, 78.60 and 78.69 % for salicylic acid at 200 ml/l over the control in 1st and 2nd seasons, respectively.

The stimulatory effects of chitosan on root growth may be due to its stimulatory influences on uptake and availability of nutrients to give healthy plants. These results are in line with those stated by Salachna *et al*. (2017) on *Verbena bonariensis*. Likewise, Baldotto *et al*. (2012) suggested that the application of HA at the indicated doses accelerates rooting ability (root length and fresh and dry matter of root) of croton and hibiscus and contributes to the formation of vigorous plants. Esringü *et al*. (2015) found that humic acid treatment was the most effective in particular on root length, fresh root weight and dry root weight of *Impatiens walleriana* when compared with control plant. However, it promotes root growth via improving better structure of soil texture and increasing soil microorganism, cell enlargement and water status necessary for vigorous root growth (Malekpoor *et al*., 2016). Also, Gad *et al*. (2016) found that spraying the plants with salicylic acid at 400 ppm increased fresh and dry weight of roots of *Ixora coccinea* plants compared to control.

Table 2. Effect of humic acid (HA), salicylic acid (SA) and chitosan (Ch) on root length (cm) as well as root fresh and dry weights per plant (g) of *Duranta erecta* var. *variegata* plant during 2017 and 2018 seasons

Treatments	Root length (cm)		Root fresh weight/plant (g)		Root dry weight/plant (g)	
	2017 season	2018 season	2017 season	2018 season	2017 season	2018 season
Control	24.07j	25.02j	10.58g	11.27g	2.29d	2.44d
2 ml/l HA	29.17h	30.50h	11.77efg	12.43efg	2.71d	2.86d
4 ml/l HA	33.83f	35.47f	13.42cd	14.33cd	3.42c	3.65c
6 ml/l HA	40.40c	42.66c	15.38ab	16.28ab	4.26a	4.51a
100 mg/l SA	26.33i	27.87i	11.44fg	12.10g	2.49d	2.63d
150 mg/l SA	31.23g	32.70g	12.45def	13.29def	3.44c	3.67c
200 mg/l SA	38.27d	40.11d	14.53bc	15.52bc	4.09ab	4.36ab
100 mg/l Ch	35.93e	37.83e	13.40cde	14.13cde	3.77bc	3.97bc
200 mg/l Ch	42.77b	45.01b	15.33ab	16.23ab	4.17ab	4.42ab
300 mg/l Ch	44.67a	46.74a	16.27a	17.43a	4.50a	4.82a

Means in the same column followed by the same letters are not significantly different according to Duncan's multiple test at ($p \leq 0.05$).

Chemical constituents:

It is quite clear from data in Table (3) that, the best treatments for improving the total content of chlorophyll (a +b) were chitosan at the rates of 300 and 200 mg/l, humic acid at rate of 6ml/l and salicylic acid at 200 mg/l. These treatments recorded 1.319 and 1.383, 1.272 and 1.331, 1.187 and 1.242 as well as 1.132 and 1.183 (mg/g fresh weight) in the 1st and 2nd seasons, respectively. In addition, all treatments under study significantly increased the percentage of the mineral contents of N, P and K in duranta plant leaves in both seasons compared with the untreated plants (control). The simulative influences on chlorophyll a+b content, total nitrogen percentage, total phosphorus percentage and potassium percentage due to Ch, SA and HA treatments application is referred to their promote effect on photosynthesis and cell enlargement and especially foliar spraying chitosan which enhances the uptake of nutrients necessary for such process. Furthermore, El-Sayed *et al*. (2016) indicated that treated Cycas plant with humic acid at 5ml/l increased the leaf content of chlorophyll a and b over control in the two seasons. Also, El-Khateeb *et al*. (2018) on *Spathiphyllum wallisii* mentioned that most of the bio-stimulants treatments significantly increased the chylorophyll a, and chitosan at 0.2 g/l induced the maximum content of chlorophyll b. Similarly, Jaddo and Rabee (2016) on *Catharanthus roseus* plants pointed out that foliar application of salicylic acid and humic acid increased nitrogen, phosphorus and potassium contents in the leaves.

Table 3. Effect of humic acid (HA), salicylic acid (SA) and chitosan (Ch) on Total chlorophyll content as well as nitrogen, total phosphorus and potassium percentages in leaves of *Duranta erecta* var. *variegata* plant during 2017 and 2018 seasons

Treatments	Total chlorophyll content (mg/g as fresh weight)		Total nitrogen (%)		Total phosphorus (%)		Potassium (%)	
	2017 season	2018 season	2017 season	2018 season	2017 season	2018 season	2017 season	2018 season
Control	0.827j	0.866j	1.42j	1.49j	0.351j	0.370j	1.64i	1.70i
2 ml/l HA	0.922h	0.965h	1.66h	1.73h	0.408h	0.430h	1.85h	1.92h
4 ml/l HA	1.031f	1.084f	1.99f	2.08f	0.484f	0.509f	2.08c	2.19f
6 ml/l HA	1.187c	1.242c	2.43c	2.53c	0.597c	0.630c	2.45c	2.59c
100 mg/l SA	0.886i	0.930i	1.55i	1.62i	0.374i	0.395i	1.83h	1.93h
150 mg/l SA	0.985g	1.029g	1.82g	1.91g	0.447g	0.471g	2.02g	2.12g
200 mg/l SA	1.132a	1.183d	2.28d	2.38d	0.552d	0.582d	2.37d	2.49d
100 mg/l Ch	1.076e	1.132e	2.14e	2.25e	0.513e	0.540e	2.25e	2.36e
200 mg/l Ch	1.272b	1.331b	2.63b	2.76b	0.615b	0.469b	2.56b	2.70b
300 mg/l Ch	1.319a	1.383a	2.89a	3.03a	0.644a	0.679a	2.64a	2.76a

Means in the same column followed by the same letters are not significantly different according to Duncan's multiple test at ($p \leq 0.05$).

CONCLUSION

From present results and under the same conditions, it can be recommended that spray *Duranta erecta* var. *variegata* plant with chitosan at the rates of 300 and 200 mg/L, humic acid at 6ml/l and salicylic acid at rate of 200mg/l to improve plant growth, root vigor and total chlorophyll content.

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تحسين النمو، وصفات الجذور وبعض المكونات الكيميائية للدورنتا المبرقشة باستخدام حمض الهيوميك وحمض الساليسيليك والشيتوزان

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أجريت هذه الدراسة في البيت المحمي بمحطة بحوث البساتين، المنصورة، مصر خلال الموسمين المتتاليين لعامي 2017 و2018. كان الهدف من الدراسة الحالية هو تحسين نمو النبات، وصفات الجذور و المكونات الكيميائية لنبات زهرة السماء (الدورانتا المبرقشة) بإستخدام حمض الهيوميك(HA) بتركيزات 2 و 4 و 6 مللي/لتر و حمض الساليسيليك(SA) بتركيزات 100 و 150 و 200 ملليجم/لتر و كذلك الشيتوزان (CH) بتركيزات 100 و 200 و 300 ملليجم/لتر. أوضحت النتائج المتحصل عليها أن معاملات حمض الهيوميك و حمض الساليسيليك و الشيتوزان بأي تركيز أثرت معنويا في تحسين نمو النبات (ارتفاع النبات، عدد الافرع لكل نبات، الاوزان الطازجة و الجافة لكل نبات و مساحة الورقة) وصفات الجذور (طول الجذر و الاوزان الطازجة و الجافة للجذور لكل نبات) مقارنة بالكنترول.أيضا، أدت جميع المعاملات قيد الدراسة الي زيادة معنوية للمحتوي الكلي من الكلوروفيل، و النسب الكلية لكل من النيتروجين و الفسفور و البوتاسيوم في أوراق النباتات مقارنة بنباتات بالكنترول(النباتات غير المعاملة). وكانت افضل النتائج في هذا الصدد (نمو النبات، الصفات الجذرية و المكونات الكيميائية) هي المتحصل عليها من المعاملتين 300 و 200 ملليجم من الشينوزان/لتر، تليهما معاملة 6 مللي من جمض الهيوميك/لتر ثم معاملة 200 ملليجم من حمض الساليسيليك/لتر في كلا الوسمين.