

ORIGINAL ARTICLES

Growth, flowering and chemical constituents of *Chrysanthemum indicum* L. plant in response to different levels of humic acid and salinity

¹Azza A.M. Mazhar, ²Shaymaa I. Shedeed, ¹Nahed G. Abdel-Aziz, ¹Mona H. Mahgoub

¹Ornamental Plants and Woody Trees Department, National Research Centre, Dokki, Giza, Egypt.

²Plant Nutrition Department, National Research Centre, Dokki, Giza, Egypt.

ABSTRACT

Humic acid compound contains many nutrient elements which improve soil fertility buildings and enhance plant growth as well as reducing the negative effects caused by salinity. Therefore a green house experiment was conducted throughout two successive seasons (2009/10 and 2010/11) to find out how far humic acid can reduce the negative effects of salinity on growth, flowering and chemical composition of chrysanthemum plants. The plants were grown to maturity in pots filled with clay soils and were subjected to four salinity levels (0, 2000, 4000, 6000 mg L⁻¹) combined with foliar application of four humic acid solutions (0, 1.0, 1.5 and 2 %) which applied two time every season. Application of saline water alone led to significant reductions in all vegetative growth and flowering parameters. The same trend was also observed concerning total carbohydrates and protein as well as N, P and K concentrations while proline and Na increased as a result of increasing salinity. On the other hand, all aforementioned growth parameters except for the content of proline and Na tended to increase by humic acid application. The interaction effect between salinity and humic acid was significant; growth parameters of the plants treated with humic acid were significantly higher under all salinity levels. These results indicates that improving nutritional status of chrysanthemum plants grown under saline condition was effective in reducing the negative effects induced by salinity.

Key words: *Chrysanthemum indicum* - Salinity – humic acid

Introduction

Chrysanthemums, often called mums or chrysanth, are of genus (*Chrysanthemum*) constituting approximately 30 species of perennial flowering plants in the family Asteraceae which is native to Asia and northeastern Europe. *Chrysanthemum indicum* L. are herbaceous perennial plants growing to 50 – 150 cm tall, with deeply lobed leaves with large flower heads that are generally white, yellow or pink in the wild. Chrysanthemum plants have been shown to reduce indoor air pollution by the NASA clean air study (Wolverton *et al.*, 2007). Extracts of chrysanthemum plants (stem and flower) have been shown to have a wide variety of potential medicinal properties, including anti-HIV-1, (Hu *et al.*, 1994 and Collins *et al.*, 1997) antibacterial (Sassi *et al.*, 2008) and antimycotic (Marongiu *et al.*, 2009).

Soil salinity is one of the most important problems in dry and semi dry climate areas of the world (Maas and Grattan, 1999). One-third of the world's arable land has been affected by soil salinity (Lazof and Berstein, 1999). Salts tend to accumulate in soil surface due to intensive evaporation conditions and insufficient leaching process (De Pascale and Barbieri, 1997). Accumulated salts deteriorate some soil physical and chemical properties. Salinity is a major abiotic stress reducing the yield of wide variety of crops all over the world (Tester and Davenport, 2003). Higher salt concentration in soil prevents plant growth (Mer *et al.*, 2000) and growing plants can be died by the excess salt concentration (Donahue *et al.*, 1983). Salt stress in plants influence some basic plant metabolic process such as, photosynthesis, energy and lipid metabolism and protein synthesis (Parida and Das, 2005). Salinity adversely affects plant by inducing injury, inhibiting growth, altering in plant morphology and anatomy, often being a prelude to tree mortality (Kozłowski and Pallardy, 1997). Nahed *et al.*, (2011) on *Matthiola incana* and Azza *et al.*, (2011) on *Schefflera arboricola* L. reported that, stem length, stem diameter and dry matter were decreased by increasing salinity stress.

Humankind has realized for thousands of years that dark-colored soils with high humus content are more fertile than light-colored soils. It has long been recognized that humic substances have many beneficial effects on soils and consequently on plant growth. Humic substances are the most widely distributed organic products of biosynthesis on the face of the earth, exceeding the amount of carbon contained in all living organisms by

Corresponding Author: Azza A.M. Mazhar, Ornamental plants and Woody trees Department, National Research Centre, Dokki, Giza, Egypt.

approximately one order of magnitude. The major functional groups of humic acid include carboxyl, phenolic hydroxyl, alcoholic hydroxyl, ketone and quinoid (Russo and Berlyn, 1990).

The mechanism of humic acid in promoting plant growth is not completely known. However, increasing cell membrane permeability, oxygen uptake, respiration, photosynthesis, phosphate uptake and root cell elongation of plant growth factors have been proposed by some authors to explain positive effect of humic acid (Cacco and Dell Agnolla, 1984 and Russo and Berlyn, 1990). In addition, humic acid has beneficial effects on nutrient uptake by plants and was particularly important for transportation and availability of micronutrient (Böhme and Thi lua, 1997).

Some researchers indicated that humic acid can be used as a growth regulator to regulate hormone level, improve plant growth and enhance stress tolerance (Piccolo *et al.*, 1992). Humic acid may stimulate shoot and root growth, and improve resistance to environmental stress in plant, but the physiological mechanisms has not been well established (Delfine *et al.*, 2005). Türkmen *et al.*, (2004 a, b) reported that humic acid may promote much growth of seedlings in salty condition.

Therefore, the objective of this study was to investigate the response of *Chrysanthemum indicum* plants grown under saline conditions to foliar application of humic acid to gauge proper nutritional management for saline soils.

Material and Methods

The experiment was carried out at the greenhouse of National Research Centre, Cairo during two successive seasons of 2009/10 to 2010/11. The main objective of this study was to investigate the different levels of humic acids application to decrease the negative effect of salinity on growth, flowering and some chemical constituents of *Chrysanthemum indicum* L.. The soil was clay in texture and composed of sand 55.77%, silt 5.35%, clay 38.88% with pH 7.63, EC 0.74 dS m⁻¹, CaCO₃ 2.3%, organic matter 1.51%, Ca 2.9, Mg 0.3, Na⁺ 2.3, K⁺ 1.2, Cl⁻ 2.5, SO₄⁻ 2.6 mg Kg⁻¹ soil. The analysis carried out according to the methods described by Chapman and Pratt (1961).

Uniformly shaped *Chrysanthemum indicum* L. cv. Zambawela (yellow flowers) were cultivated in pot 30 cm diameter filled with 10 kg of soil that had passed through 2 mm sieved. The seedlings were planted in early September 2009 and 2010. The plants were under normal photoperiods at September, Oct., Nov., Dec., Jan., Feb. and March. And the plants were under normal temperature (outdoor conditions) through both seasons. The experiment included 16 treatments which were the combinations of four salinity levels (0, 2000, 4000 and 6000 mg L⁻¹) and foliar application four of humic acid solution (0, 1.0, 1.5, 2%). The available commercially fertilizer used through this experimental work was Kristalon (NPK 19:19:19) produced Phayzon Company, Holland. The fertilizer rate (5.0 g/pot) used in four doses after 4, 8, 16 and 20 weeks from sowing seedlings were irrigated for 4 weeks with tap water. Then, three salinity levels were prepared (2000, 4000 and 6000 ppm) by adding a mixture of sodium chloride and calcium chloride at a ratio (1:1) by weight for irrigating seedlings. Tap water was used for control 2 L of water (either fresh or salinity water) was added to each pot every three days through the course of this study (7 months). The potassium humate used in this study is produced by alkaline treatment of Victoria brown coal and is commercially available in Australia (18% K-humate). Plants were sprayed with twice freshly prepared solution of potassium humate at (1.0, 1.5 and 2.0%) in addition to the untreated plants which were sprayed with tap water. The plants treated with K-humate two times of 30 days intervals starting on 15th November in both seasons. A completely randomized design was used, each treatment was replicated six times. Plant height (cm), number of branches/plant, root length (cm), stem diameter (cm), number of flowers, flower diameter (cm), pedicel length (cm) and fresh and dry weight of all plants organs (roots, shoots and flowers) (g) estimated at the end of experiment. Total carbohydrates percentage was determined according to the method of Dubios *et al.*, (1956). The proline concentration was determined using fresh material according to Bates *et al.*, (1973). Nitrogen percentage was determined by the modified micro Kjeldahl method as described by Pregl (1945). Protein percentage was estimated by multiplying percentage of nitrogen by the factor 6.25 according to A.O.A.C. (1980). Phosphorus, potassium and sodium were determined according to the method described by Cottenie *et al.*, (1982). The obtained results were subjected to statistical analysis of variance according to the method described by Snedecor and Cochran (1980) and the combined analysis of the two seasons was calculated according to the method of Steel and Torrie (1980).

Results and Discussion

Growth Characters:

Vegetative growth characters were significantly decreased by increase salinity levels compared to control, where no salts were added (Table 1). Plant height, stem diameter, root length and number of branches per plant were decreased by (5.36, 17.40 and 27.83%), (5.08, 14.82 and 21.77%), (5.00, 13.33 and 45.98%) and (5.85,

17.15 and 25.52%), respectively as a results of irrigation with saline waters having the concentrations of 2000, 4000 and 6000 mg L⁻¹, respectively compared with control. The depressive effect on plant height by salinity might be mainly attributed to reduction in cell division and enlargement, water stress induced by salinity, also causes of stomata which reduced the supply of carbon dioxide of photosynthesis. In this respect, (Lewis *et al.*, 1980) reported that natural hormones might be affected due to the saline conditions leading to unbalanced growth of the plant, consequently, the decrease in number of branches / plant. Holloway and Alston (1992) mentioned that increasing salinity level decreased water permeability and osmotic potential. The depressive effect of high salinity levels on the above mentioned plant traits are in parallel with those of El-Khateeb *et al.*, 2010; El-Dabh *et al.*, 2011 ; Azza *et al.*, 2011.

The obtained data in Table (2) also cleared that, shoots and roots fresh and dry weight were significantly depressed gradually by increasing salinity levels. This effect was pronounced in plants grown under higher salinity level (6000 mg L⁻¹). Shoots and roots fresh weights were decreased by 37.17 and 35.20% respectively, while shoots and roots dry weight were decreased by 40.40 and 39.54% respectively, compared to plants received non saline water.

The depressive effect of salinity on fresh and dry weight of shoots and roots might be attributed to the inhibitory effects induced by salinity on many metabolic processes including enzyme activities, protein and nucleic acid synthesis and chloroplasts. Ashraf and O'leary (1996) pointed out that CO₂ uptake was decreased by increasing salinity level, and the decrease in CO₂ uptake was parallel by a reduction in transpiration and stomatal conductance. They also suggested that the change in stomatal resistance under saline conditions may be responsible for reducing photosynthesis and water use efficiency. Similar findings were also registered by Nahed *et al.*, (2011) on *Matthiola incana* and Azza *et al.*, (2011) on *Schefflera arboricola*.

On the other hand, foliar application of humic acid had a significant stimulatory effect on growth parameters of *Chrysanthemum indicum* plants. Humic acid solution of 2 % was the most effective treatment which had the highest values of plant height, stem diameter, root length and number of branches / plant. The increments were 46.39, 39.53, 139.47 and 50.52% respectively, compared with untreated plants. Shoots and roots fresh and dry weights were significantly increased by increasing the concentration of humic solution (Table 2). The highest values of shoots and roots fresh weight were obtained when plants treated with 2.0 % humic acid solution. The increments were (104.9, and 86.4 %) respectively, while shoots and roots dry weight were increment by (124.0 and 104.7%) respectively, compared with untreated plants. The use of humic acid has been reported to have beneficial effects on plants. This result may be due to the role of humic acid as a nutrient supplying which increase soil fertility and increase the availability of nutrient elements as reported by David *et al.*, (1994). Chen and Aavid (1990) reported that humic substances have very pronounced influence on the growth of plant roots and enhance root initiation which known root stimulator. Humic acid improve growth of plant foliage and roots. Vaughan (1974) proposed that, humic acids may primarily increase root growth by increasing cell elongation or root cell membrane permeability, therefore increased water uptake by increased plant roots. It can produce root systems with increased branching and number of fine roots, as a result potentially increase nutrients uptake by increase root surface area (Rauthan and Schnitzer, 1981).

The interaction between different treatments (salinity + humic acid) were almost significant for vegetative growth characters. The highest values due to the irrigation with salinity water + humic acid due (2000 ppm salinity + 2.0% humic acid) for stem diameter, fresh and dry weights of shoots. While the interaction between untreated plants and humic acid at 2.0% gave the highest values of other parameters. These results are inline with those reported by Chen and Aviad (1990), Cimrine *et al.*, (2001). It is possible that the positive effect of humic acids on plant growth could be mainly due to hormone like actives which were reported by Piccolo *et al.*, (1992).

Flowering Characters:

Increasing the levels of salinity from 2000 to 6000 ppm significantly decreased all flowering characters (number of flowers / plant, flower diameter (cm), pedicel length (cm), fresh and dry weight of flowers (g)) compared with control plants (Tables 3,4). The lowest values of flowering parameters were obtained from plants treated with 6000 ppm salinity. The decrements were (32.55, 11.53, 24.94, 49.62 and 46.76%) respectively, compared with control plants. This reduction in flowering parameters may be due to the inhibition of photosynthesis of plants via the changes of chlorophyll contents and components and damage of photosynthetic apparatus (Lyengar and Reddy, 1996). It also inhibits the photochemical activities and decreases the activities of enzymes in the Calvin cycle (Sairam and Tyagi, 2004). This result is in line with Zapryanova and Atonassova (2009) on *Tagetes* and *Ageratum* plants and Nahed *et al.*, (2011) on *Matthiola incana* plants.

All flowering characters followed the same trend of the growth parameters. Generally, humic acid caused an increase in flowering parameters. Furthermore, the application of humic acid at 2.0% gave the highest values of growth parameters. The increment were 82.06, 19.64, 50.17, 163.58 and 209.66% from number of flowers / plant, flower diameter, pedicel length, fresh and dry weight of flowers respectively, compared with untreated

plants. This result may be due to the role of humic acid as a source of nutrients and increasing the soil fertility which consequently increased the growth of flowers of *Chrysanthemum indicum* plants. Our results here in harmony with those gained by Abd El- Al *et al.*, 2005.

The results of the effect of the interaction between salinity and humic acid on flowers characters presented in Tables (3,4). These results indicated that the highest number of flowers / plant, flower diameter, pedicel length, fresh and dry weight of flowers were obtained from untreated plants & humic acid at 2.0% followed by low level of salinity (2000 ppm) and humic acid at 2.0%.

Chemical Constituents:

Total carbohydrates:

Total carbohydrates were gradually decreased as salinity of the irrigation water increased (Fig. 1). The reduction in total carbohydrates as salinity levels increased may be related to respiration processes since the free sugar was the main sugar pattern involve in the mechanism of respiration (Bernstein *et al.*, 1972). However, the observed reduction in total carbohydrates content in shoots and roots resulting from salinity treatments go in line with that was found by many authers in increasing total carbohydrates content as a result of using different salinity levels in many plants (Azza *et al.*, (2011) on *Schefflera arboricola* L. and Nahed *et al.*, (2011) on *Matthiola incana* plants.

On the other hand, foliar application of humic acid increased total carbohydrates of shoots and roots of the plants compared with untreated plant and the best result was obtained with 2% humic acid solution. Data emphasized that total carbohydrates content increased when plants treated with humic acid, the highest value of this parameter was obtained plants treated with humic acid at 2.0 %, compared with control plants. Chen and Avid, (1990) pointed out that, humic acid aid in correcting plant chlorosis, thus enhancement of photosynthesis density. These increments lead to positive effects on growth parameters and increased total carbohydrates content of plants. These results were in accordance with those obtained by Sahar *et al.*, (2009).

The interaction between two factors (salinity x humic acid) showed an increase in total carbohydrates percentage compared with salinity treatments.

Proline Content:

Data in Fig. (2) illustrated that, in both seasons, salinity induced a marked increase in proline concentration in different organs of *Chrysanthemum indicum* plant (shoots and roots), this increase was positively correlated to salt levels. In this connection, AcKerson, (1984) stated that osmotic adjustment within the cytoplasm is maintained by synthesis of compatible solutes, some of which such as proline have deleterious effects on metabolism and growth at high concentrations. Moreover, Bellinger *et al.*, (1991) proposed that the increase of in free proline in salt-stressed plant tissues could be interpreted as a tolerance mechanism of osmotic regulation and / or accumulation of the excess of ammonium produced by salt stress. Similar result was also obtained by Azza *et al.*, (2011) on *Schefflera arboricola* plant and Nahed *et al.*, (2011) on *Matthiola incana* plants.

Data also clearly show that, proline content was significantly reduced by increasing the level of humic acid. This result may be due to the role of humic acid in increasing the soil content of organic matter which reduces the negative effect of salts, also proline increase with increasing salinity as reaction to resist salinity (Erik *et al.*, 2000).

The interaction between humic acid and salinity was significantly increased under medium and high salinity levels when sprayed with 1.0 and 1.5 % humic acid solutions.

Protein Content:

Protein content followed the same trend obtained previously in total carbohydrates and was also gradually decreases by increasing the salinity level. In this regard, Larsen (1982) mentioned that salinity depressed protein content, which might occur through a faint incorporation of acid and / or protein hydrolysis which might cause accumulation of toxic products.

Meanwhile, protein content gave the highest values by higher levels of humic acid. The increased in protein content of *Chrysanthemum indicum* plants due to humic acid application, these increments might have influenced plant growth directly through its effects on ion uptake or by the effects on growth regulators. These results were in line with those obtained by Sivakuman and Devarajan (2005).

As for the interaction between salinity and humic acid application, the highest values of protein content were observed in plants irrigated with 2000 mg L⁻¹ and sprayed with 2.0 and 1.5% humic acid solutions respectively.

Minerals composition:

Figs. (4-7) salinity treatments decreased leaf N, P and K concentrations due to the fact that water stress inhibits plant growth through inhibition of various physiological and biochemical process including photosynthesis, respiration, hormones, nutrient uptake and metabolism (Kramer and Boyer, 1995). These results are in agreement with those obtained by Azza *et al.*, (2008) on *Taxodium disticum* plant and Nahed *et al.*, (2011) on *Matthiola incana* plants.

In this connection, Hanafy Ahmed (1996) pointed out that salinization impaired N accumulation and incorporation into protein and raised total free amino acid accumulation in plant growth under saline conditions. Also, it can be suggested that amino acids can act as components of salt tolerance mechanism and build up a favorable osmotic potential inside the cell in ordered to compact the effects of which replaced nitrate in the vacuoles. The reduction in P uptake under saline conditions could be explained on the fact that high concentration of Ca in saline soils decreased P solubility combined with salinity induced decreased root growth. Jungk (1991), reported that when plants grown in soils, any reduction in root elongation will reduce the available P to the plants since P movement in the soil towards the roots is mainly by diffusion and higher P concentration may needed to supply the total demand. In respect, it is interesting to note that the change in Na⁺ concentration in every plant organs due to chloride salinity levels showed completely an opposite picture to that previous reported fork. Actually such results reflect to great extent the competition between the uptake of the two cations i.e. Na⁺ and K⁺. Such competition might be due to the existence of general carrier for their absorption by the roots. The metabolism of Na⁺ increases and K⁺ decreases in plants stressed by salt (De Lacerda *et al.*, 2003).

The results in the same Figs showed that, all minerals content (N, P and K) under investigation were gradually increased by increasing humic acid concentrations, but Na content was decreased. These results were in line with those obtained by Sivakumar and Devarajan, 2005 and Sahar *et al.*, 2009. These increments led to positive effect on growth parameters and increased nitrogen and phosphorus concentrations. Additionally, humic acid maintained high level of acid phosphate activity which led to increase phosphate activity holds for increased phosphorus uptake by plants (Malcum and Vanghan, 1979). Humic acid have been reported to enhance mineral nutrients uptake by plants, because it affects the permeability of membranes of roots (Mesut *et al.*, 2010).

The results also showed that, adding different levels (1.0, 1.5 and 2.0 %) of humic acid could alleviate the harmful effect of salinity. These results might be due to the fact that humic substances are renowned for their ability to: chelate soil nutrients, improve nutrient uptake especially phosphorus, sulfur and nitrogen, stimulate soil biological activity and act as a storehouse of N, P, S and Zn (Richard, 2004).

Therefore, it can be concluded that foliar application of humic acid to the plants grown in region irrigated with saline water could alleviate salinity stress of *Chrysanthemum indicum* plants seedlings.

Table 1: Effect of different levels of Humic acid on plant height (cm), stem diameter (mm), root length (cm) and number of branched/plant *Chrysanthemum indicum* plant irrigated with different concentrations of salinity. (Average of two seasons 2009/2010 and 2010/2011).

| Humic acid % (B) | Salinity ppm (A) | | | | | | | | | | | | | | | | | | | |
|------------------|-------------------|--------|--------|--------|--------|--------------------|------|------|------|------|------------------|-------|-------|-------|-------|----------------------------|-------|-------|-------|-------|
| | Tap water | 2000 | 4000 | 6000 | Mean | Tap water | 2000 | 4000 | 6000 | Mean | Tap water | 2000 | 4000 | 6000 | Mean | Tap water | 2000 | 4000 | 6000 | Mean |
| | Plant height (cm) | | | | | Stem diameter (cm) | | | | | Root length (cm) | | | | | Number of branches / plant | | | | |
| Tap water | 103.30 | 97.55 | 92.53 | 88.5 | 95.45 | 0.46 | 0.44 | 0.42 | 0.40 | 0.43 | 15.50 | 14.00 | 12.30 | 11.00 | 13.20 | 42.00 | 40.33 | 39.00 | 37.67 | 39.75 |
| 1.0 | 145.50 | 136.70 | 122.33 | 108.67 | 128.30 | 0.61 | 0.58 | 0.53 | 0.48 | 0.55 | 32.33 | 29.50 | 21.23 | 17.13 | 25.05 | 63.67 | 58.33 | 51.33 | 45.00 | 54.58 |
| 1.5 | 148.33 | 140.30 | 127.70 | 114.43 | 132.69 | 0.63 | 0.60 | 0.55 | 0.49 | 0.57 | 34.45 | 31.00 | 24.45 | 18.23 | 27.03 | 64.33 | 60.00 | 52.67 | 46.33 | 55.83 |
| 2.0 | 158.00 | 152.25 | 130.20 | 118.60 | 139.76 | 0.63 | 0.66 | 0.56 | 0.51 | 0.60 | 41.85 | 37.50 | 26.40 | 20.67 | 31.61 | 69.00 | 66.33 | 55.00 | 49.00 | 59.83 |
| Mean | 138.76 | 131.70 | 118.19 | 108.55 | 123.76 | 0.60 | 0.57 | 0.52 | 0.47 | 0.56 | 31.03 | 28.00 | 21.10 | 16.76 | 23.98 | 59.75 | 56.25 | 49.50 | 44.50 | 51.40 |
| LSD 0.05 | | | | | | | | | | | | | | | | | | | | |
| A | 2.46 | | | | | 0.03 | | | | | 1.10 | | | | | 1.40 | | | | |
| B | 2.46 | | | | | 0.03 | | | | | 1.10 | | | | | 1.40 | | | | |
| A * B | 4.90 | | | | | 0.05 | | | | | 2.20 | | | | | 2.79 | | | | |

Table 2: Effect of different levels of Humic acid on shoot fresh weight (g), shoot dry weight (g), Root fresh weight (g) and Root dry weight (g) *Chrysanthemum indicum* plant irrigated with different concentrations of salinity. (Average of two seasons 2009/2010 and 2010/2011).

| Humic acid % (B) | Salinity ppm (A) | | | | | | | | | | | | | | | | | | | |
|------------------|------------------------|--------|--------|--------|--------|----------------------|-------|-------|-------|-------|-----------------------|-------|-------|-------|-------|---------------------|-------|-------|-------|-------|
| | Tap water | 2000 | 4000 | 6000 | Mean | Tap water | 2000 | 4000 | 6000 | Mean | Tap water | 2000 | 4000 | 6000 | Mean | Tap water | 2000 | 4000 | 6000 | Mean |
| | Shoot fresh weight (g) | | | | | Shoot dry weight (g) | | | | | Root fresh weight (g) | | | | | Root dry weight (g) | | | | |
| Tap water | 133.87 | 120.41 | 110.32 | 98.75 | 115.84 | 36.37 | 33.87 | 30.22 | 27.36 | 31.96 | 28.00 | 25.47 | 22.20 | 20.45 | 24.03 | 10.02 | 9.04 | 7.88 | 7.14 | 8.52 |
| 1.0 | 260.01 | 229.04 | 187.70 | 142.62 | 204.84 | 79.51 | 67.12 | 51.64 | 41.43 | 59.93 | 51.72 | 42.20 | 35.26 | 30.58 | 39.94 | 20.33 | 16.20 | 13.08 | 11.07 | 15.17 |
| 1.5 | 252.66 | 240.57 | 194.15 | 160.58 | 211.99 | 74.88 | 72.01 | 57.99 | 45.73 | 62.65 | 48.27 | 46.25 | 37.26 | 32.88 | 41.17 | 18.83 | 17.90 | 14.08 | 12.00 | 15.70 |
| 2.0 | 273.22 | 285.53 | 214.77 | 175.88 | 237.35 | 84.23 | 89.77 | 62.99 | 49.37 | 71.59 | 54.06 | 52.04 | 39.10 | 34.00 | 44.80 | 21.62 | 20.66 | 14.90 | 12.58 | 17.44 |
| Mean | 229.94 | 218.89 | 176.44 | 144.46 | 204.84 | 68.75 | 65.69 | 50.71 | 40.97 | 56.25 | 45.50 | 41.49 | 33.46 | 29.48 | 36.98 | 17.70 | 15.95 | 12.94 | 10.70 | 14.40 |
| LSD 0.05 | | | | | | | | | | | | | | | | | | | | |
| A | 6.20 | | | | | 2.40 | | | | | 1.22 | | | | | 0.99 | | | | |
| B | 6.20 | | | | | 2.40 | | | | | 1.22 | | | | | 0.99 | | | | |
| A * B | 12.38 | | | | | 4.79 | | | | | 2.44 | | | | | 1.98 | | | | |

Table 3: Effect of different levels of Humic acid on number of flowers / plants, flower diameter (cm) and pedicel length of flower (cm) *Chrysanthemum indicum* plant irrigated with different concentrations of salinity. (Average of two seasons 2009/2010 and 2010/2011).

| Humic acid % (B) | Salinity ppm (A) | | | | | | | | | | | | | | |
|------------------|---------------------------|-------|-------|-------|-------|----------------------|------|------|------|------|-------------------------------|------|------|------|------|
| | Tap water | 2000 | 4000 | 6000 | Mean | Tap water | 2000 | 4000 | 6000 | Mean | Tap water | 2000 | 4000 | 6000 | Mean |
| | Number of flowers / plant | | | | | Flower diameter (cm) | | | | | Pedicel length of flower (cm) | | | | |
| Tap water | 22.33 | 21.33 | 18.36 | 16.67 | 19.68 | 7.40 | 7.25 | 7.10 | 6.97 | 7.18 | 6.00 | 5.80 | 5.68 | 5.57 | 5.76 |
| 1.0 | 37.00 | 33.50 | 29.00 | 25.00 | 31.13 | 8.70 | 8.50 | 7.93 | 7.60 | 8.18 | 8.20 | 7.67 | 7.25 | 6.10 | 7.31 |
| 1.5 | 37.67 | 34.50 | 30.67 | 25.67 | 32.13 | 8.83 | 8.63 | 8.30 | 7.79 | 8.39 | 8.80 | 8.00 | 7.30 | 6.37 | 7.62 |
| 2.0 | 43.33 | 41.33 | 31.33 | 27.33 | 35.83 | 9.15 | 8.95 | 8.37 | 7.37 | 8.59 | 10.50 | 9.63 | 7.35 | 7.10 | 8.65 |
| Mean | 35.08 | 32.67 | 26.34 | 23.66 | | 8.52 | 8.32 | 7.93 | 7.56 | | 8.38 | 7.78 | 6.90 | 6.29 | |
| LSD 0.05 | | | | | | | | | | | | | | | |
| A | 1.10 | | | | | 0.72 | | | | | 0.62 | | | | |
| B | 1.10 | | | | | 0.72 | | | | | 0.62 | | | | |
| A * B | 2.19 | | | | | 1.44 | | | | | 1.24 | | | | |

Table 4: Effect of different levels of Humic acid on number of flowers / plants, flower diameter (cm) and pedicel length of flower (cm) *Chrysanthemum indicum* plant irrigated with different concentrations of salinity. (Average of two seasons 2009/2010 and 2010/2011).

| Humic acid % (B) | Salinity ppm (A) | | | | | | | | | |
|------------------|-------------------------|-------|-------|-------|-------|-----------------------|-------|-------|------|-------|
| | Tap water | 2000 | 4000 | 6000 | Mean | Tap water | 2000 | 4000 | 6000 | Mean |
| | Flower fresh weight (g) | | | | | Flower dry weight (g) | | | | |
| Tap water | 35.62 | 29.55 | 26.16 | 21.79 | 28.28 | 6.48 | 5.32 | 4.66 | 3.81 | 5.07 |
| 1.0 | 84.63 | 68.85 | 55.00 | 38.89 | 61.84 | 18.11 | 14.11 | 10.73 | 7.19 | 12.54 |
| 1.5 | 80.06 | 72.96 | 58.03 | 42.16 | 63.30 | 16.89 | 15.18 | 11.08 | 7.93 | 12.77 |
| 2.0 | 99.77 | 86.87 | 63.24 | 48.30 | 74.54 | 21.95 | 18.84 | 12.77 | 9.23 | 15.70 |
| Mean | 75.02 | 64.55 | 50.61 | 37.79 | | 15.86 | 14.12 | 9.81 | 7.04 | |
| LSD 0.05 | | | | | | | | | | |
| A | 1.32 | | | | | 0.72 | | | | |
| B | 1.32 | | | | | 0.72 | | | | |
| A * B | 2.65 | | | | | 1.45 | | | | |

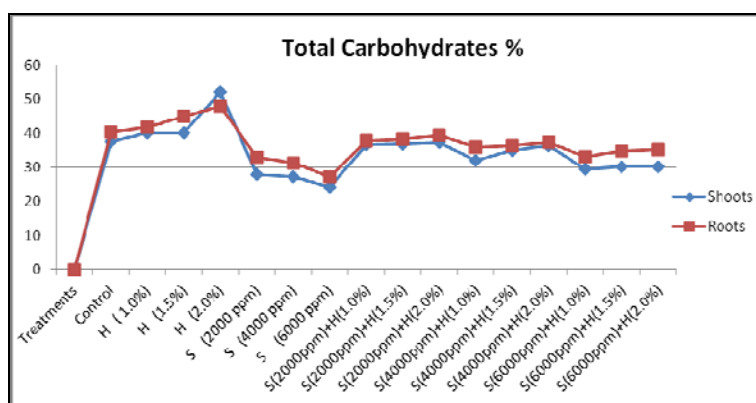


Fig. 1: Effect of different levels of Humic acid on total carbohydrates of *Chrysanthemum indicum* plant irrigated with different concentrations of salinity. (Average of two seasons 2009/2010 and 2010/2011).

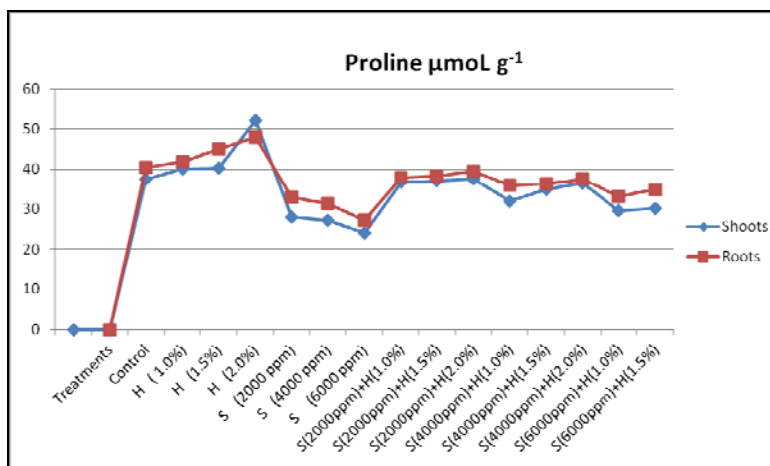


Fig. 2: Effect of different levels of Humic acid on Proline $\mu\text{mol g}^{-1}$ % of *Chrysanthemum indicum* plant irrigated with different concentrations of salinity. (Average of two seasons 2009/2010 and 2010/2011).

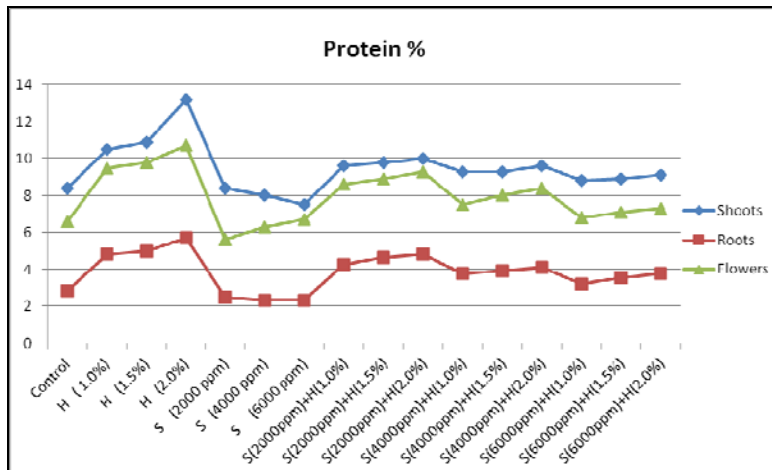


Fig. 3: Effect of different levels of Humic acid on Protein % of *Chrysanthemum indicum* plant irrigated with different concentrations of salinity.(Average of two seasons 2009/2010 and 2010/2011).

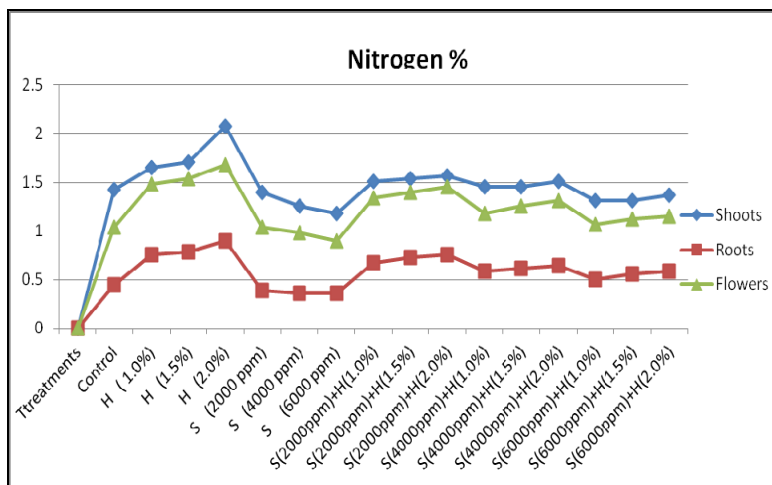


Fig. 4: Effect of different levels of Humic acid on Nitrogen % of *Chrysanthemum indicum* plant irrigated with different concentrations of salinity.(Average of two seasons 2009/2010 and 2010/2011).

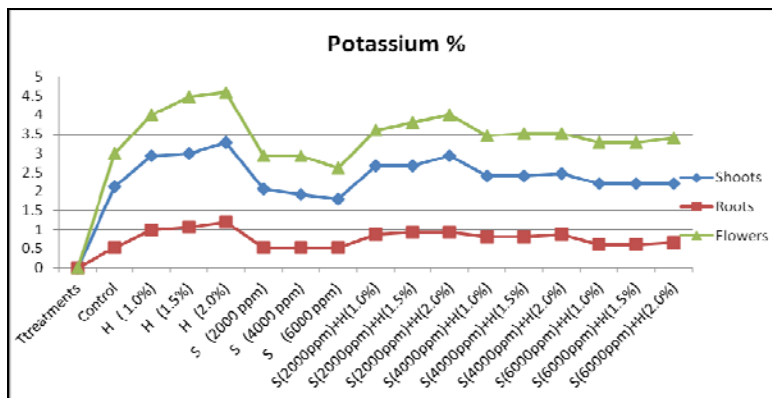


Fig. 5: Effect of different levels of Humic acid on Potassium % of *Chrysanthemum indicum* plant irrigated with different concentrations of salinity. (Average of two seasons 2009/2010 and 2010/2011).

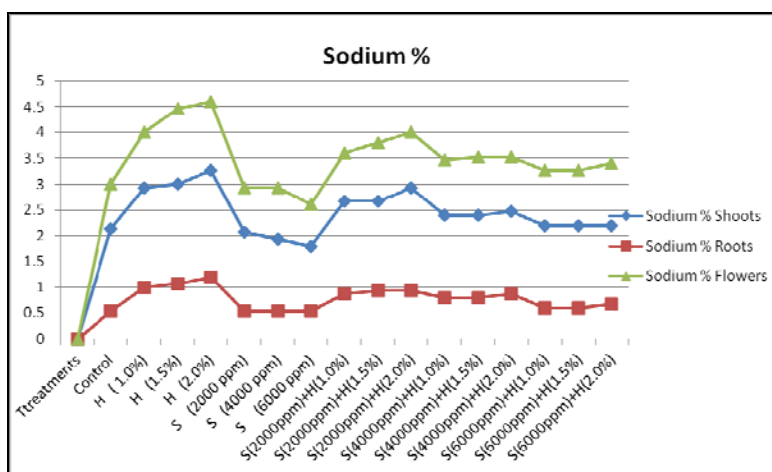


Fig. 6: Effect of different levels of Humic acid on Sodium % of *Chrysanthemum indicum* plant irrigated with different concentrations of salinity. (Average of two seasons 2009/2010 and 2010/2011).

References

- Abd El-Al, Faten, M.R. Shafeek, A.A. Ahmed and A.M. Shaheen, 2005. Response of growth and yield of onion plants to potassium fertilizer and humic acid. *J. Agric. Sci. Mansoura Univ.*, 30(1): 441-452.
- A.O.A.C., 1980. Official Methods of Analysis of Association of official Analytical Chemists 12th Ed. Washington, P.C.
- Ashraf, M. and T.W. O'leary, 1996. Responses of some newly developed salt tolerant genotypes of spring wheat to salt stress, yield components and ion distribution. *Journal of Agronomy and Crop Science*, 179(2): 91-101.
- Azza, A.M. Mazhar, M. Sahar Zaghloul and T. El-Mesiry, 2008. Nitrogen forms effects on the growth and chemical constituents of *Taxodium disticum* grown under salt conditions. *Australian J. Basic and Applied Sci.*, 2(3): 527-534.
- Azza, A.M. Mazhar, Mon. H. Mahgoub and Nahed G. Abd El-Aziz, 2011. Response of *Schefflera arboricola* L. to gypsum and sulphur application irrigated with different levels of saline water. *Australian Journal of Basic and Applied Sciences*, 5(10): 121-129.
- Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of proline for water stress studies. *Plant and Soil*, 39: 305-307.
- Bellinger, Y., A. Bensaoud and F. Larher, 1991. Physiological significance of proline accumulation, a trait of use to breeding for stress tolerance. In :Acevedo *et al.*, (eds.): *Physiology – Breeding of winter cereals for stressed Mediterranean Environment*, INRA, Paris, pp: 449-458.
- Bernstein, L., L.E. Francois and R.A. Clark, 1972. Salt tolerance of ornamental shrubs and ground covers. *J. Amer. Soc. Hort. Sci.*, 97: 550-556.
- Bohme, M. and H. Thi Lua, 1997. Influence of mineral and organic treatments in the rizosphere on the growth of tomato plants. *Acta Hort.*, 450: 161-168.
- Cacco, G. and G. Dell Agnolla, 1984. Plant regulator activity of soluble humic substances. *Can. J. Soil Sci.*, 64: 25-28.
- Chapman, H.D. and P.F. Pratt., 1961. *Methods of Analysis for Soils, plant and water*. Div. of Agric. Sci. Univ. of Calif., pp: 309.
- Chen, Y. and T. Avaid, 1990. Effect of humic substances on plant growth. Pp. 161-186. In: American Society of Agronomy and Soil Science Society of America (eds.). *Humic substances in soil and crop science; selected readings*. American Society of Agronom, Madison, WI.
- Cimrin, K.M., D. Karaca and M.A. Bozkurt, 2001. The effect of NPK and humic acid applications on growth and nutrition of corn plant (*Zea mays* L.) Ankara University *J. Agric. Sci.*, 7: 95-100.
- Collins, R.A., T.B. Ng, W.P. Fong, C.C. Wan and H.W. Yeung, 1997. A comparison of human immunodeficiency virus type 1 inhibition by partially purified aqueous extracts of Chinese medicinal herbs. *Life Sciences*, 60(23): 45-51.
- Cottenie, A., M. Verloo, L. Kiekens, G. Velghe and R. Camerlynck, 1982. *Chemical Analysis of Plant and Soil*. Laboratory of Analytical and Agrochemistry, state Univ. Ghent Belgium, pp: 100-129.
- David, P.P., P.V. Nelson and D.C. Sanders, 1994. A humic acid improves growth of tomato seedling in solution culture. *J. Plant Nutr.*, 17(1): 173-184.

- DeLacerda, C.F., J. Cambraia, M.A. Oliva, H.A. Ruiz and J.T. Prisco, 2003. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. *Environ. Exp. Bot.*, 49: 107-120.
- Delfine, S., R. Tognetti, E. Desiderio and A. Alvino, 2005. Effect of foliar application of N and humic acid on growth and yield of durum wheat. *Agron. Sustain. Dev.*, 25: 183-191.
- DePascal, S. and G. Barbieri, 1997. Effect of soil salinity and top removal on growth and yield of broad bean as green vegetable. *Scientia Hort.*, 71: 147-165.
- Donohue, R.L., R.V. Miller and J.C. Shickluna, 1983. *Soils, an introduction to soils and plant growth*. Prentice Hall, Englewood cliffs, NJ.
- Dubios, M., K.A. Gilles J.K. Hamilton, P.A. Robers and P.A. Smith, 1956. A colorimetric for determination of sugar and related substances. *Anal., Chin.*, 28: 350-356.
- El-Dadh, R.S., M.A. El-Khateeb, A.A.M. Mazher and A.A. Abd El-Badaie, 2011. Effect of salinity on growth and chemical constituents of *Moringa oleifer* LAM. *Bull. Fac. Agric., Cairo Univ.*, 62: 378-386.
- El-Khateeb, M.A., A. Nabih, A.A. Nasr and H.S.M. Hussien, 2010. Growth and chemical constituents of *Ceiba pentandra* L. plant in response to different levels of saline irrigation water. *Bull. Fac. Agric. Cairo Univ.*, 61: 214-221.
- Erik, B.G., Feibert, G. Clint Shock and D. Lament Saundres, 2000. Evaluation of humic acid and other non ventional fertilizer additions for onion production. *Malheur Experiment station Oregon State Univeristy. Ontario, OR*.
- Hanafy Ahmed, A.H., 1996. Physiological studies on tiploun and nitrate accumulation in lettuce plants. *J. of Agric. Sci., Mansoura Univ.*, 21: 3971-3994.
- History of Chrysanthemum. (<http://www.mums.org/journal/articles/chrysanthemum-history.htm>) National Chrysanthemum Society USA.
- Holloway, R.E. and A.M. Alston, 1992. The effects of salt and boron on growth of wheat Australian. *J. of Agric. Res.*, 43(5): 987-1001.
- Hu, C.Q., K. Chen, Q. Shi, R.E. Kilkuskie, Y.C. Cheng and K.H. Lee, 1994. Anti-AIDS agents, 10. Acacetin-7-O-beta-D-galactopyranoside, an anti-HIV principle from *Chrysanthemum morifolium* and a structure-activity correlation with some related flavonoid. *Journal of Natural Products*, 57(1): 42-51.
- Jungk, A., 1991. Dynamics of nutrients movement at the soil root interface. pp: 455-481. In: Y. Waisel, A. Eshel, and U. Kafkafi (eds), *Plant Roots, The Hidden Half*. Marcel Dekker, Inc. New York, NY.
- Kozlowski, T.T. and Pallardy, 1997. *Physiology of woody plants*. 2nd Edn. Academic press, San Diego. Calif., USA.
- Kramer, P.J. and J.S. Boyer, 1995. *Water relations of plants and soils*. Academic Press, San Diego, California.
- Larsen, K.L., 1982. Drought injury and resistance of crop plants. Department of Agronomy of Missouai (C.F. Physiological Aspect of Dryland Farming. Gupta, U. S. 1982, Oxford & IBM, Publishing Co. New Delhi Bombay, Calcutta).
- Lazof, D.B. and N. Berstein, 1999. The NaCl inhibition of shoot growth: The case for disturbed nutrition with special consideration of calcium. *Adv. Bot. Res.*, 29: 113-189.
- Lewis, O.A.M., E.O. Leldi and S.H. Lips, 1980. Effect of irrigation source on growth response to salinity stress in maize and wheat. *New. Physiol.*, 411(2): 155-162.
- Lyengar, E.R. and M.P. Reddy, 1996. Photosynthesis in highly salt tolerant plants. In: Pesserkali, M.(Ed), *Handbook of photosynthesis*. Marshal Dekar, Baten Rose, USA, pp: 897-909.
- Maas, E.V. and S.R. Grattan, 1999. Crop yields as affected by salinity. In: *Agron. Monogr. No. 38* (Eds.: R. W. Skaggs and J. van Schilfgaarde). J. Am. Soc. Agron., Modison, WI, 55-108.
- Malcum, R.L. and D. Vaughum, 1999. Humic substances and phosphatase activities in plant tissues. *Soil Biochem.*, 11: 253-259.
- Marongiu, B., A. Piras and S. Porcedda, 2009. Chemical and biological comparisons on supercritical extracts of *Tanacetum cinerariifolium* (Trevir) Sch. Bip. with three related species of chrysanthemums of Sardinia (Italy National Product Research, 23(2): 190-199.
- Mer, R.K., P.K. Prajith, D.H. Pandya and A.N. Pandey, 2000. Effect of salts on germination of seeds and growth of young plants of *Hordeum vulgare*, *Triticum aestivum*, *Cicer arietinum* and *Brassica juncea*. *J. Agron. Crop Sci.*, 185: 209-217.
- Mesut, C.K., T. Onder, T. Metin and T. Burcu, 2010. Phosphorus and humic acid application alleviate salinity stress of pepper seedling. *African Journal of Biotechnology*, 9(36): 5845-5851.
- Nahed, G. Abd El-Aziz, Azza, A.M. Mazhar and Mona, H. Mahgoub, 2011. Influence of using organic fertilizer on vegetative growth, flowering and chemical constituents of *Matthiola incana* plant grown under saline water irrigation. *World Journal of Agricultural Sciences*, 7(1): 47-54.
- Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: A review. *Ecotoxicol. Environ. Safety*, 60: 324-329.

- Piccolo, A., S. Nardi and G. Concheri, 1992. Structural characteristics of humic substance as related to nitrate uptake and growth regulation in plant systems. *Soil Biol. Biochem.*, 24: 373-380.
- Pregl, F., 1945. *Quantitative Organic Micro Analysis*. 4th Ed., J. and A Churchill, La London.
- Rauthan, B.S. and M. Schnitzer, 1981. Effect of fulvic acid on the growth and nutrient content of cucumber (*Cucumis sativus*) plants. *Plant and Soil*, 63: 491-495.
- Richard, L., 2004. Humic substances in biological agriculture. ACRES USA. A voice for Eco-Agriculture, 34(1&2).
- Russo, R.O. and G.P. Berlyn, 1990. The use of organic biostimulants to help low input sustainable agriculture. *J. Sustain. Agric.*, 1: 19-42.
- Sahar, M. Zaghoul, E.M. Fatma, El-Quesni and Azza, A.M. Mazhar, 2009. Influence of potassium humate on growth and chemical constituents of *Thuja orientalis* L. seedlings. *Ozean Journal of Applied Science*, 2(1): 73-78.
- Sairam, R.K. and A. Tyagi, 2004. Physiology and molecular biology of salinity stress tolerance in plants. *Current Sci.*, 86(10): 408-421.
- Sassi, A.B., F. Harzallah-Skhiri, N. Bourgougnon and M. Aouni, 2008. Antimicrobial activities of four Tunisian *Chrysanthemum* species. *The Indian Journal of Medical Research*, 127(2): 92-183.
- Sharama, S., 1995. Studies on growth, water relations and distribution of Na⁺, K⁺ and other ions in wheat under short-term exposures to salinity. *Indian Journal of Plant Physiology*, 38(3): 233-235.
- Sivakumar, A. and L. Devarajan, 2005. Nitrogen of K-hamates on the yield and nutrient uptake of Rice. *Madras Agric. J.*, 92: 718-721.
- Snedecor, G.W. and W.G. Cochran, 1980. *Statistical Methods*, 7th ed. Iowa State Univ., Press Amer, Iowa, USA.
- Steel, R.G.D. and J.H. Torrie, 1980. *Principles and Procedures of statistics*. Mc grow-hill book Co., Inc., New-york. Toronto, London.
- Tester, M. and R. Development, 2003. Na⁺ tolerance Na⁺ transport in higher plants. *Annal Bot.*, 91: 503-527.
- Turkmen, O., A. Dursun, M. Turan and C. Erdinc, 2004a. Calcium and humic acid affect seed germination, growth and nutrient content of tomato (*Lycopersicon esculentum* L.) seedling in saline soil conditions. *Acta Agric. Scand., Sect. B, Soil Plant Sci.*, 54: 168-174.
- Turkmen, O., M.A. Bozkurt, M. Yildiz and K.M. Cimrin, 2004b. Effect of nitrogen and humic acid applications on the head weight, nutrient and nitrate contents in lettuce. *Adv. Food Sci.*, 26: 1-6.
- Vaughan, D., 1974. Possible mechanism for humic acid action on cell elongation in root segments of *Pisum sativum* under aseptic conditions. *Soil Biol. Biochem.*, 6: 241-247.
- Vaughan, D., R.E. Malcolm and B.G. Ord, 1985. Influences of humic substances on biochemical process in plant. In : Vaughan, D. and R.E. Malcolm eds. *Soil organic matter and biological activity*. Martinus Nijoff, Dr. Jjunk, Dordrecht, Netherlands.
- Wolverton, B.C., Rebecca C. McDonald and E.A. Watkins, Jr, 2007. Foliage plants for removing indoor air pollutants from Energy-efficient Homes. <http://ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/19860066312-1986066312.pdf>.
- Zapryanova, N. and B. Atanassova, 2009. Effect of salt stress on growth and flowering of ornamental Annual spp. *Biotechnol & Biotechnol EQ*, 23: 177-180.