Influence of diphenylamine and Ascorbic acid on the production of *Dendranthema grandiflorum, Ram*.

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Abstract: This investigation was carried out during 2012 and 2013 seasons on *Dendranthema grandiflorum*, Ram. grown in 25 cm diameter pots at a commercial nursery in Damanhour City, El-Beheira Governorate, Egypt. The aim of this work was to study the effect of different levels of diphenylamine at rates of (zero, 150, and 200 ppm) and ascorbic acid at rates of (zero, 150, and 250 ppm) on the vegetative growth, flowering and chemical constituents of *Dendranthema grandiflorum, Ram*. From the obtained results it was concluded that treating chrysanthemum plants with diphenylamine at 150ppm and ascorbic acid at 250ppm improve the vegetative growth, flowering characteristics and the contents of chlorophyll "A" and "B", total carotenoids, total soluble sugars and total free amino acids in the leaves of chrysanthemum plants.
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Key words: *Dendranthema grandiflorum*, diphenylamine, ascorbic acid.

Introduction:

Dendranthema grandiflorum the queen of Autumn's flowers is a perennial plant belonging to family Compositae (Asteraceae).It is commonly believed that the plant was first grown in Japan, it was in China that the plant was first cultivated, ancient records show that it was mentioned by Confucius about 500 years B.**C (Jwoolman, 1989)** *Dendranthema* may have a single, double, in curving reflexing and other forms of flower and it may be controlled to many types of growth. *Dendranthema* soon rated among the top flowers for decorative purposes, they can add tremendously to the beauty of house and garden, they have so many types and colours, there is something to suit all tastes and requirements. They are the most keeping flowers for home use and one of the most adaptable to design work. They are used as potted plants, as greenhouse subject or for growing in window boxes. They have additional advantage of flowering at a time of year when there are very few other flowers in the gardens. Every year there are flower fairs in Cairo and Alexandria especially in November for the Queen of Autumn's flower (chrysanthemum).

Antioxidants are substances which can prevent the oxidative damage of the cell. When cells use oxygen, they naturally produce free radicals (by -products) which can cause damage. Antioxidants act as "free radical scavengers" and hence prevent and repair damage done by the other free radicals. Antioxidants such as ascorbic acid and diphenylamine have different biological and physiological roles, which reflect on different physiological processes in the plant **(Naglaa *et al.*, 2011)**. Diphenylamine is an aromatic antioxidant amine, a plant growth regulator and a fungicide with the structural formula of $(C_6H_5)_2$ NH, Diphenylamine is used as a parent compound for vulcanization accelerators and for antioxidants for rubber industry. These derivatives are added to rubber products to retard degradation by oxidants and ozone. They are intermediates for the production of dyes, pH indicators, polymers and fibers, and curing agents, Diphenylamine is used as a test for oxidizing agents and it detecting DNA in labs .Moreover, it is used to absorb decomposed catalysts in solid fuel rocket propellant **(Carrasco *et al.*, 2005)**.

Ascorbic acid ($C_6H_8O_6$) is a water soluble vitamin and an essential nutrient found mainly in fruits and vegetables. Ascorbic acid is known as a growth regulator factor that influences many biological processes. **(Price, 1976)** reported that ascorbic acid increased nucleic acid content especially RNA. It also influenced the synthesis of enzymes, nucleic acid and protein. In addition, it acts as coenzyme in metabolic changes **(Patil and Lall, 1973 and Reda *et al.*, 1977)**. Some research works have been carried out on the use of diphenylamine and ascorbic acid in the production of ornamental plants. **(Abdel – Aziz *et al.*, 2009)** who added ascorbic acid as a foliar spray on Gladiolus plants at the rates of 50, 100 and 200 ppm. They found that, the maximum plant height, number of leaves, fresh weight of leaves and dry weight of leaves were obtained by using ascorbic acid at 100 ppm, while using ascorbic acid at 200 ppm gave the maximum number and fresh weight of cormlets, spike length, number of florets and fresh and dry weight of the florets. **(El-Quesni *et al.*, 2008)** studied the effect of foliar spray of ascorbic acid at 0, 50 and 100 ppm on the growth of *Hibiscus rosasinensis*. They found that,

most criteria on vegetative growth expressed as plant height, number of branches and leaves / plant, stem diameter, leaf area, fresh and dry weights of plant organs were significantly affected by application of ascorbic acid especially at 100 ppm. Also, using ascorbic acid at 100 ppm significantly increased number of flowers / plant, as well as fresh and dry weights of flowers, as compared with control plants.

(Eid *et al.*, 2010) studied the effect of foliar application of various concentrations (50, 100 and 150 ppm) of ascorbic acid, thiamin and α- tocopherol separately or collectively on some flower characters (flower yield and weight of flowers) on *Jasminum grandiflorum* plants. The results reveled that all treatments of ascorbic acid, thiamine and α - tocopherol significantly increased flower yield / plant and weight of flowers on *Jasminum grandiflorum* plants, compared with untreated plants. The highest increase of flowers were observed in plants treated with ascorbic acid 100ppm + thiamine 100 ppm + α- tocopherol 100 ppm.

(Abou Dahab and Abdel-Aziz, 2006) reported that, the foliar spraying of diphenylamine at 50 or 100 ppm significantly increased plant growth of *Philodendron* erubescens plants. (in terms of plant height, number of leaves / plant, stem diameter, root length, leaf area, as well as fresh and dry weights of the different plant parts). The maximum plant growth (as determined by all the recorded parameters) was obtained from plant treated with diphenylamine at the rate of 100 ppm.

(Eman *et al.*, 2012) found that treating gladiolus plants with diphenylamine at 150 ppm and ammonium nitrate at 6 gm / plant improved the vegetative growth, flowering characteristics, corms production and total chlorophyll contents in the leaves of gladiolus plants.

The objective of this work was to study the effect of different levels of diphenylamine and ascorbic acid and their combinations on the vegetative growth, flowering and some chemical analysis of *Dendranthema grandiflorum*, Ram.

2. Material and methods:

The present study was carried out in the two successive seasons: 2012 and 2013 at a commercial nursery in Damanhour City, El-Beheira Governorate, Egypt. The rooted cuttings of *Dendranthema grandiflorum* were planted in 10cm diameter pots. One rooted cutting was planted in each pot using a clay soil, on March 15th, 2012 and repeated during the same date in the second season. After three months on 15th June the plants were transplanted to the soil pots of 25 cm diameter. The analysis of the soil medium presented in Table (1) was carried out in the soil testing laboratory, Faculty of Agriculture, Alexandria University, in Alexandria. There are 9 treatments which are all the possible combinations of the three levels concentrations of diphenylamine (Zero, 150, and 200 ppm) and ascorbic acid (Zero, 150, and 250 ppm). These concentrations were sprayed early in the morning four times (at 30, 45, 60, and 75 days from the planting) on the plant foliage until the run off point.

The plants were fertilized with a complete chemical fertilizer of N: P_2O_5: K_2O (7: 7: 7) at rate of 5gm per pot. The first addition of fertilizer was done after 6 weeks from the final planting, and then repeated each two weeks until the appearance of flowers buds as reported by **(Post, 1989)**. The following data were recorded; plant height(cm), leaves area per plant (cm^2) according to **(Zidan, 1962)**, leaves fresh weight (g), flowering time (days), number of flowers, flowering duration (days), chlorophyll A, B, and carotenoids contents using the method described by **(Moral and Porath, 1980).**

Leaf sample were dried and their content of the total soluble sugars were determined according to **(Dabois *et al.*, 1956)**. The content of total free amino acids in leaves was determined according to **(Rosein, 1957)**.

The experimental design was a complete randomized block design in a factorial experiment with three replicates, each replicate contained three plants. Data were subjected to analysis of variance (ANOVA) using the SAS program, **(SAS institute 2002)** and the mean values were compared using ref tukey's test at L.S.D0:05 level **(Snedecor and Cochran, 1974).**

Table (1): The chemical properties of clay soil

pH	EC (ds/m^{-1})	N (%)	P (%)	K (%)
7.2	4.33	0.81	0.123	0.37

3. Results and Discussion

Vegetative growth:

Plant height (cm): The analysis of variance showed that the F-values of different levels of diphenylamine (D) were not significant in the two experiment seasons, while the F-values of different levels of Ascorbic acid (A) not significant in the first season but significant in the second season. The interaction between the diphenylamine and ascorbic acid were significant in the first and second seasons. The tallest plant (48.87 cm =

means of the two seasons) was found by using diphenylamine at 150ppm combined with ascorbic acid at 250 ppm. These results may be due to the synergistic effects of the two materials at proper concentrations on plant growth as a result of their involvement in the main metabolic processes especially of carbohydrate, metabolism improved biosynthesize activity and DNA replication which led to enhance of cell division and cell enlargement as reported by **(Bartoli *et al.*, 1999)**. These results are in harmony with those obtained by **(Abou Dahab and Abd El Aziz, 2006)**.

Leaves area (cm^2): Data presented in (Table, 2) showed that the highest significant increase in leaves area per plant was obtained by using diphenylamine at 200 ppm combined with ascorbic acid at 150 ppm during the two seasons. These results may be due to the increase in leaves expansion and size or both as a result, the leaves area could be increased **(Naglaa, *et al.*, 2011)**. **(Russel, 1982)** indicated that the increase in growth as a result of application of amino acid may be due to their conversion into IAA. The results are agreement with those reported by **(Eman *et al.*, 2012)**.

Leaves fresh weight (g): The data recorded in the two seasons showed that the diphenylamine and ascorbic acid increased the fresh weight of leaves compared with the control (Table, 2). The most effective treatment was the application of diphenylamine at 150 ppm and ascorbic acid at 250 ppm. This may be attributed to the role of each material on activation the vegetative growth. Ascorbic acts as co- enzyme in the enzymatic reactions by which carbohydrates, fats and proteins as reported by **(Helsper *et al.*, 1982)**. The diphenylamine may stimulate growth by increasing the leaves development and the size of photosynthesizing surface. Consequently, using the two materials combined at the suitable concentrations gave the highest accumulations of the matter of the leaves. These results are in agreement with those obtained by **(Gomma, 2003, Talaat *et al.*, 2005and Naglaa *et al.*, 2011).**

Number of lateral branches: Data in (Table, 3) show that the maximum increase in the number of branches per plant was found by using diphenylamine at 150 ppm combined with ascorbic acid at 250 ppm during the two seasons. Similar trend of results was obtained by **(Abou Dahab and Abdel-Aziz, 2006 and El-Quesni *et al.*, 2008)**.

Flowering characteristics:

Flowering time (days): Generally, data of means of flowering time of the two seasons in (Table, 3) Clear that using any level of the diphenylamine or ascorbic acid alone led to a significant reduction in the number of days needed for flowering compared with the control treatment. Furthermore, the application of diphenylamine at 150 ppm and ascorbic acid at 250 ppm gave the minimum time taken for flowering of *Dendranthema* compared with other treatments in the two seasons. These results may be due to that presence of any of the used material can serve as a source of energy and enhance the synthesis of auxins, organic materials or enzymes **(Patil and Lall, 1973 and John *et al.*, 1997)** These results agree with those obtained by **(Naglaa *et al.*, 2011)**.

Number of Flowers: Data in (Table, 3) indicated that using diphenylamine at 150 ppm combined with ascorbic acid at 250 ppm gave the highest increase in the number of flowers during the two seasons. These results may be due to the effect of the used materials on improving the vegetative growth of chrysanthemum plants, consequently the production and accumulation of the biosynthesizes would be increased, thus more flowers could be initiated and developed on the plant. These results are in harmony with those obtained be **(Naglaa *et al.*, 2011)**.

Flowering duration (days): The data recorded in the two seasons showed that adding diphenylamine at 150 ppm and ascorbic acid at 250 ppm led to an increase in the flowering duration compared with the control (table, 4). These results may be due to that using diphenylamine and ascorbic acid at suitable concentrations act as scavengers, helping to prevent cell and tissue damage and delay of the flowers senescence **(Eman *et al.*, 2012).**

Chemical constituents:

Chlorophyll content (A and B): Data presented in (table, 4) indicated that, the addition of the different combination between the two experimental factors led to increase in the chlorophyll "A" and chlorophyll "B" content of the leaves of chrysanthemum plants, Also using diphenylamine at 150 ppm combined with ascorbic acid at 250 ppm improve the content of chlorophyll "A" and chlorophyll "B" in the leaves. These results may be attributed to the used materials have the ability on keeping the chlorophyll from degradation and delaying its senscence, consequentally the chlorophyll "A" and "B" in *Dendranthema* leaves could be increased. The present data are in harmony with **(Shoala, 2000, Abou Dahab and Abdel-Aziz, 2006 and Naglaa *et al.*, 2011)**

Total carotinoides content (mg / 100g of leaves fresh weight): The recorded data revealed that plants treated with diphenylamine at 200 ppm and ascorbic acid at 150 ppm had the highest carotenoids content, compared to that found in plants receiving any other treatment. The present data are in agreement with previous reports of **(Shoala, 2000 and Naglaa *et al.*, 2011)** they reported that foliar application of amino acid caused an increase in the contents of photosynthetic pigments.

Total soluble sugars contents: Data in (table, 5) indicated that application of the used materials diphenylamine and tryptophan as a foliar spray caused a significant increase in the contents of total soluble

Table (2): Means of plant height (cm), leaves area per plant (cm2), and leaves fresh weight (gm) of *Dendranthema grandiflorum*, Ram. as influenced by the different levels of diphenylamine (D), ascorbic acid (A) and their interaction (D×A) in the two seasons of 2012 and 2013

Dipheneylamine (ppm)	Ascorbic acid (ppm)				Ascorbic acid (ppm)			
	0	150	250	Mean	0	150	250	Mean
	2012 Season				2013 Season			
	Plant height (cm)				Plant height (cm)			
0	39.10	45.07	42.17	42.11B	39.10	45.90	42.27	42.42B
150	44.23	45.94	47.63	46.12A	46.70	44.80	48.87	46.79A
200	46.20	46.43	45.77	46.13A	46.57	47.27	46.50	47.11A
Mean	43.17 B	45.81A	45.41A	44.79	44.12C	45.73B	46.47A	45.44
L.S.D $_{0.05}$For (D×A)	1.46				1.52			
	Leaves area (cm^2)				Leaves area (cm^2)			
0	133.67	281.00	214.87	209.84C	132.17	284.03	215.03	210.41C
150	206.37	229.00	222.77	219.38B	214.70	229.80	222.73	222.41B
200	216.73	305.73	269.23	263.90A	212.40	308.60	266.67	275.89A
Mean	185.59C	271.91A	235.62B	231.04	199.77C	274.14A	234.81B	236.24
L.S.D $_{0.05}$For (D×A)	26.78				27.98			
	Leaves fresh weight (g)				Leaves fresh weight (g)			
0	27.87	32.13	31.69	30.56B	27.93	32.00	31.68	30.54B
150	31.57	35.50	37.81	31.67B	33.63	35.67	37.89	35.25A
200	34.63	35.88	34.52	35.62A	34.96	35.93	34.86	35.73A
Mean	28.65B	34.71A	34.50A	32.62	32.17B	34.81A	34.53A	33.84
L.S.D $_{0.05}$For (D×A)	5.28				2.58			

$L.S.D_{0.05}$ = least significant differences at 0.05 probability
Diphenylamine (D), ascorbic acid (A)

Table (3): Means of number of lateral branches, flowering time (days), and number of flowers of *Dendranthema grandiflorum*, Ram. as influenced by the different levels of diphenylamine (D), ascorbic acid (A) and their interaction (D×A) in the two seasons of 2012 and 2013

Dipheneylamine (ppm)	Ascorbic acid (ppm)				Ascorbic acid (ppm)			
	0	150	250	Mean	0	150	250	Mean
	2012 Season				2013 Season			
	Number of lateral branches				Number of lateral branches			
0	7.89	11.45	9.85	9.73B	8.00	11.33	10.00	9.78B
150	10.78	12.33	13.81	12.31A	11.00	12.33	13.89	12.41A
200	11.99	13.33	13.17	12.83 A	12.00	13.24	13.13	12.78A
Mean	10.22C	12.86A	11.78B	11.62	10.33B	12.78A	11.86A	11.65
L.S.D $_{0.05}$For (D×A)	2.74				2.26			
	Flowering time (days)				Flowering time (days)			
0	95.33	84.33	89.33	91.33A	100.67	84.33	90.00	91.67A
150	96.33	87.00	81.67	88.33B	97.00	86.00	81.67	88.22B
200	83.00	89.67	93.33	88.67A	83.67	89.33	93.00	87.67B
Mean	92.11A	88.44B	87.78B	89.44	90.11A	89.11B	88.22B	89.16
L.S.D $_{0.05}$For (D×A)	3.41				2.67			
	Number of flowers				Number of flowers			
0	30.33	35.33	30.33	32.00B	30.00	36.33	30.33	32.22B
150	35.33	31.67	43.00	36.67A	35.00	31.67	43.33	36.67A
200	38.67	36.67	36.00	37.22A	38.33	36.67	36.33	37.11A
Mean	34.78B	36.78A	34.33B	35.29	34.44B	36.78A	34.78B	35.33
L.S.D $_{0.05}$For (D×A)	1.65				2.63			

$L.S.D_{0.05}$ = least significant differences at 0.05 probability
Diphenylamine (D), ascorbic acid (A)

sugars in the leaves. The recorded data also revealed that plants treated with diphenylamine at 150ppm combined with ascorbic acid at 250 ppm had the highest soluble sugars content, compared to that found in plants receiving any other treatment. These results are in agreements with those obtained by **(Nahed *et al.*, 2009)** and **(Talaat *et al.*, 2002)**.

Total free amino acids: The results recorded in (table, 5) showed that spraying chrysanthemum plants with different treatments caused increase in the content of total free amino acids in leaves. The increase in the content of total free amino acids as a result of the ascorbic acid and diphenylamine treatments may be attributed to its conversion of to IAA, as stated by **(Phillips, 1971)**. Furthermore, the combination between the two amino acids were almost positive for the content of the total free amino acids, Diphenylamine at 150 ppm + ascorbic acids at 250 ppm gave the highest values of the total free amino acid. These results are in agreement with the findings **of (Abou Dahab and Abdel-Aziz 2006 and Nahed *et al.*, 2009)**

In conclusion

It could be recommended that treatment of *Dendranthema grandiflorum* plants with diphenylamine and ascorbic acid (especially diphenylamine at 150ppm + ascorbic acid at 250ppm) are considered to be of a great beneficial effect on plant growth and chemical constituents.

Table (4): Means of flowering duration (days), chlorophyll "A" (mg/100g of fresh leaves), and chlorophyll "B" (mg/100g of fresh leaves), of *Dendranthema grandiflorum*, Ram. as influenced by the different levels of diphenylamine (D), ascorbic acid (A) and their interaction (D×A) in the two seasons of 2012 and 2013

Dipheneylamine (ppm)	Ascorbic acid (ppm)				Ascorbic acid (ppm)			
	0	150	250	Mean	0	150	250	Mean
	2012 Season				2013 Season			
	flowering duration (days)				flowering duration (days)			
0	23.33	28.00	28.00	26.44B	23.00	27.67	28.33	26.33B
150	24.33	24.67	31.67	26.89AB	24.67	24.67	32.00	27.11A
200	28.33	30.33	24.33	27.67A	28.00	30.33	24.00	27.44A
Mean	25.33B	28.00A	27.67A	27.00	25.22C	28.11A	27.55B	26.96
L.S.D $_{0.05}$For (D×A)	3.28				2.87			
	chlorophyll "A" (mg/100g of fresh leaves)				chlorophyll "A" (mg/100g of fresh leaves)			
0	45.85	63.19	65.21	61.08C	55.55	62.35	65.26	61.06C
150	63.15	66.01	72.79	65.96B	62.70	66.44	74.92	65.96 B
200	68.71	62.24	66.57	67.20A	68.74	62.40	66.58	67.97A
Mean	63.60B	63.81B	66.83A	64.74	64.39B	63.73B	66.86A	64.99
L.S.D $_{0.05}$For (D×A)	4.12				2.43			
	chlorophyll "B" (mg/100g of fresh leaves)				chlorophyll "B" (mg/100g of fresh leaves)			
0	29.37	38.22	39.12	35.77B	28.38	37.95	39.83	35.39B
150	36.94	40.22	25.02	39.80A	35.98	40.14	42.34	39.45A
200	42.24	37.91	39.78	40.28A	43.59	38.93	40.72	41.08A
Mean	36.44C	38.78B	40.58A	38.61	35.99B	39.00A	40.69A	38.64
L.S.D $_{0.05}$For (D×A)	3.93				237			

$L.S.D_{0.05}$ = least significant differences at 0.05 probability
Diphenylamine (D), ascorbic acid (A)

Table (5): Means of Total carotenoides (mg/100g of leaves), Total soluble sugars (mg/g dry weight of leaves), and Total free amino acid (%) of *Dendranthema grandiflorum*, Ram. as influenced by the different levels of diphenylamine (D), ascorbic acid (A) and their interaction (D×A) in the two seasons of 2012 and 2013

Dipheneylamine (ppm)	Ascorbic acid (ppm)				Ascorbic acid (ppm)			
	0	150	250	Mean	0	150	250	Mean
	2012 Season				2013 Season			
	Total carotenoides (mg/100g of leaves)				Total carotenoides (mg/100g of leaves)			
0	133.00	151.10	159.30	147.80C	132.09	151.44	159.62	147.27C
150	151.69	160.11	145.57	152.46B	151.75	160.03	144.37	152.05B
200	150.75	165.40	172.97	163.04A	150.97	165.59	173.09	163.22A
Mean	145.15B	158.87A	159.28A	154.43	144.94B	159.02A	159.03A	154.33
L.S.D $_{0.05}$For (D×A)	4.18				6.21			
	Total soluble sugars (mg/g dry weight of leaves)				Total soluble sugars (mg/g dry weight of leaves)			
0	2.90	3.41	3.74	3.35B	2.90	3.40	3.74	3.35b
150	3.17	4.23	5.08	4.15A	3.16	4.25	5.06	4.16A
200	3.59	4.75	4.11	4.16A	3.60	4.91	4.10	4.20A
Mean	3.22B	4.52A	3.92A	3.88	3.22B	4.19A	4.30A	3.90
L.S.D $_{0.05}$For (D×A)	1.63				2.34			
	Total free amino acid (%)				Total free amino acid (%)			
0	3.36	4.50	5.79	4.55B	3.33	4.53	5.77	4.54B
150	4.36	6.22	7.59	6.07A	4.41	6.26	7.51	6.06A
200	5.65	7.16	7.45	6.75 A	5.63	7.17	7.33	6.71A
Mean	4.46C	5.96B	6.94A	5.79	4.46C	5.99B	6.87A	5.77
L.S.D $_{0.05}$For (D×A)	1.88				3.66			

L.S.D$_{0.05}$ = least significant differences at 0.05 probability
Diphenylamine (D), ascorbic acid (A)

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