Effect of Putrescine and Uniconazole Treatments on Flower Characters and Photosynthetic Pigments of *Chrysanthemum indicum* L. Plant

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Abstract: The effect of Putrescine at the concentration of 100,200 and 300 ppm and Uniconazole at 20, 40 and 60 ppm in addition to control (distilled water) on flower characters, total carbohydrates and photosynthetic pigments in flowers of Chrysanthemum plant during 2004/2005 and 2005/2006 had been evaluated studied. The obtained data indicated that all flower characters and chemical composition were significantly increased by foliar application of Putrescine at the three concentrations. Uniconazole treatments delayed start of flowering after spraying, decreased pedicle length and length of flower stalk, while it increased yield of flowers, diameter of inflorescence, vase life, total carbohydrates in the flowers and photosynthetic pigments chl. (a),(b) and carotonoids . The highest values were found when plants were treated with 200 ppm Putrescine and 20 ppm Uniconazole.
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1. Introduction:

Chrysanthemum indicum L. plant is one of the leading cut flowers and potted plants in the international market. It is a perennial herb grown in Egypt as one of the most important cut flowers and pot plants, the inflorescence consists of several ray and disc flowers (florets) and such, is called a (flower head),which is in markets because of its beautiful shape and longevity in vases. Chrysanthemum is a short day plant because of its habit of flowering only under short day conditions. A major reason being that the time of flowering can be modified throughout the year. The development of Chrysanthemum industry as a major ornamental cut flower and pot plant enterprises supports the major thrusts of the government to develop the non –traditional export products that will boost the industry to earn foreign currency.The immediate benefits from Chrysanthemum production will substitute the imported flowers with locally produced ones.

The Polyamines; namely Putrescine (Put), Spermine and Spermidine are well known as affecting different plant developmental processes (Kumar *et al*., 1997) and(Martin-Tanguy,2001). They modulate several growth and developmental processes Viz., cell division, differentiation, flowering, fruit ripening, embryogenesis, senescence and rhizogenesis. In all these plant growth processes, polyamines have been described showing various roles such as a new class of plant growth regulators, Hormonal Second Messengers (HSM) and one of the reserves of carbon and nitrogen at least in cultured tissues. At cellular pH values, these compounds behave as cations and can interact with anionic macromolecules such as DNA, RNA phospholipids and certain proteins (Hepy and Persson, 1990). PAs may act as anti-senescence agents (Galston and Kaur-Sawhey, 1995) and this property is largely based on a decrease in PA content with leaf aging and senescence in excised leaf or leaf segments. The mechanism of senescence inhibition by PAs may also be related to their possible inhibition of ethylene synthesis (Lee *et al*., 1997).This property may account for PAs acting as effective scavengers of free radical generated in a number of chemical and in vitro enzymes.

Uniconazole is one of plant growth regulators having important role in crop production, toward manipulation of plant growth and yield. Triazole plant growth regulators induce a variety of morphological and biochemical responses in plants (Fletcher and Hofstra, 1988).

The aim of this study is to investigate the effect of both Putrescine and Uniconazole on flower characters and photosynthetic pigments of Chrysanthemum *plant.*

2. Materials and Methods:

Putrescine is (tetramethylene Diamine $C_4H_2N_2$, molecular weight 88.15), (Wettable powder with 98% activity) obtained from Sigma Chemical Company and Uniconazole Sumitomo Chemical Japanese Company Limited plant growth retardant kindly, supplied by their scientific Bureau in Cairo.

The substance (Wettable powder with 10%activity) [(E)-1-(P-Chlorophenyl) 4.4-Dimethyl-2-(1,2,-4 Triazole1-1l)1-Penten-3-ol],with molecular formulae C_{15} $H_{18}Cl$ N_{3o}, molecular weight 291.78, water solubility 8.4 ppm at 25°C, solubility in methanol 20-10 (W/W% at 21°C) were used.

The plants were sprayed with bioregulators three times (1st November, 1st December and 1st January 2004/2005 and 2005/2006) with freshly prepared solutions of Putrescine (100, 200 and 300 ppm) and Uniconazole (20, 40 and 60 ppm), while the control plants were sprayed with distilled water. The experiment was a complete randomized blocks design, with six treatments plus control. Each treatment has three replicates (each replicate contain five plants). The volume of the spraying solution was maintained to cover the whole plant foliage till run off point.

The flowers were weighed, dried in an electric oven at 70 °C^o till a constant weight, ground to fine powder by an electric mill and stored for chemical analysis to estimate total carbohydrates.

Additional samples were taken after ten days from each spray to estimate flower characters (flowering start after spraying, flowers yield, inflorescence diameter, pedicle length of inflorescence, length of inflorescence stalk, vase life (days), and fresh and dry weight of inflorescence.

Total carbohydrates were determined in the dry powder of chrysanthemum flowers using colorimetric method at wave length 490 nm as described by (Herbert *et al*., 1971).

Chlorophyll (a), (b) as well as carotenoids were determined in the fresh material 10 days after each spray as described by **(**Wattestein,1957).

Statistical analysis**:** Data obtained were subjected to standard analysis of variance procedure. The values of L.S.D were obtained whenever F values were significant at 5% level, according to (Snedecor and Cochran, 1980).

3. Results and Discussion:

Flowering start after spraying

Data in Table (1) indicated that spraying Chrysanthemum plants with Putrescine at 100, 200 and 300 ppm lead to early blooming than control plants. The longest period, which was obtained in Jan. and Feb. from application of 200 ppm Putrescine, and 300 ppm in Dec. and Jan. then 100 Putrescine in Dec. in both seasons, respectively. On the other hand, spraying the plants with Uniconazole at 20, 40 and 60 ppm delayed flowering in January, February and March for the 1st and 2nd season, respectively as compared with control plants, while in December all used concentrations did not produce any flowers. It may be due to the effect of Putrescine on the IAA synthesis, which increases the synthesis of IAA enzyme and increasing the tryptophane levels, which are precursors of IAA hormone.

The results are in agreement with those obtained by(Koriesh *et al* ,1989), (Dutta *et al*.,1993) and (Kang *et al*.,1995) on Chrysanthemum. They found that growth regulators enhanced earlier flowering than untreated plants.

Yield of inflorescence

Data presented in Table (2) indicate that foliar spraying of Chrysanthemum plants with Putrescine at the rate of 100,200 and 300 ppm caused a significant increase in flower yield in both seasons than control plants.The highest values were found at 200 ppm Putrescine, giving 147and 161 for the 1st and 2nd season, respectively. Application of Uniconazole treatments at 20 ppm gave the highest values 103and 104 of inflorescence plants in the 1st and 2nd season, respectively. These results are in agreement with obtained by(Mahgoub *et al*.,2006 b) on *Dianthus caryophyllus* plants they found that Putrescine at 200 and 400 ppm caused a significant increase in flower yield as compared with untreated plants.

With regard to the effect of Uniconazole; our results are in agreement with those obtained by(Bekheta ,1992), (Wu *et al*.,1996), (Bekheta,2000) and (El-Kadey,2002) they found that using Uniconazole increased yield over than untreated plants.

Diameter of inflorescence

Data presented in Table (2) indicate that spraying Putrescine at 200 ppm significantly increased diameter of flower head, giving 8.0 cm and 8.6 cm/ inflorescence compared with control plans that gave 5.6 cm and 5.9 cm/inflorescence in the 1st and 2nd season, respectively. Other concentrations increased also the diameter of flowering heads as compared with the untreated plants, in both seasons

In case of Uniconazole application, it was found that low concentrations 2o and 40 ppm significantly increased the inflorescence diameter by 21.4 and 23.2% over than that of unsprayed plants in the 1st season. While spraying the plants with 20 ppm Uniconazole in the 2nd season gave 7.2 cm showing its significant effect, while the other rates were insignificantly effective.

Similar finding was obtained by (Haggag, 1997) on Chrysanthemum. (Dutta *et al*., 1993) on some Chrysanthemum cultivars, found that Uniconazole increased flower diameter than control plants.

Pedicle length of inflorescence

Data in Table (2) showed that foliar application of Chrysanthemum plants with Putrescine increased insignificantly pedicle length of flowers as compared with the control plants in the 1stseason. In case of the

2nd season, the application of 200 and 300 ppm significantly increased the pedicle length of flowers than application of 100 ppm Putrescine 9.8cm as compared with control plants 9.0cm in both seasons.

On the other hand, using concentrations of 40 and 60 ppm Uniconazole significantly decreased pedicle length of flowers than the control plants 8.9 and 9.0 cm for the 1st and 2nd season.

Length of inflorescence stalk

Data in Table (2) revealed that foliar spraying of Chrysanthemum plants with Putrescine significantly increased the length of flowering stalk in the two seasons. The highest value 30.6 and 32.3cm had been recorded for plants treated with 200 ppm as compared with control plants 20.7 and 22.0 cm in the 1st and 2nd season, respectively.

In case of Uniconazole; data showed that as the concentration was raised the recorded mean value for this character decreased to reach its minimum value 16.6 and 17.3 cm for the 1st and 2 nd respectively, as compared with control plants 20.7 and 22.0 cm. These results were in harmony with those obtained by (Mao *et al*., 1991) on *Salvia splendens* and (Haggag, 1997) on Chrysanthemum, who stated that PP-333 application reduced flower stalk length.

Vase life/days

Data in Table (2) indicated that the control treatment recorded 11.3 and 14.3 days for flowers vase life, while using 200 ppm Putrescine gave the longest period of flower vase life 26.0 and 27.0 days in 1st and 2nd seasons, respectively. In 1st season treated plants with 100 and 300 ppm Putrescine gave 20.7 and 24.3 days. The same trend was obtained in the 2nd season.

Application of Uniconazole increased significantly the flowers vase life. The longest vase life 19.0 and 20.0 days had been recorded for plants treated with 20 ppm in the 1st and 2nd seasons, respectively.

These results are in agreement with that obtained by(Mahgoub *et al*. ,2006 b) on *Dianthus caryophyllus* plants.

The increment of vase life of flowers due to growth regulators may be due to increasing protein content in petals and ovaries (Lukaszewska,1988), or due to cytokinins, which are able to reduce and delay the production of endogenous ethylene.

Also polyamines may retard senescence by inhibiting ethylene production (Suttle, 1981). The increment of polyamines was accompanied by inhibition of lipid peroxidation and the inhibition of lipid peroxidation may be one of the mechanisms responsible for the anti-senescence effects of polyamines. (Borrell *et al*., 1997)

Fresh weight of inflorescence

Data in Table (2) indicated that spraying the plants with Putrescine had significantly effect on the fresh weight of flowers. The highest value had been obtained from spraying the plants with 200 ppm.

Using Putrescine at the concentration of 100 ppm and 300 ppm increased fresh weight having 6.3and 7.0 g for the 1st season and 6.6 and 7.3 g for the 2nd season, respectively.

Application of 20 ppm Uniconazole increased significantly fresh weight of treated plants. The main estimated values were 5.3 and 6.3 g while the control plants, produced 4.5 and 4.6 g in both seasons, respectively. Raising the application rate to 40 ppm and 60 ppm decreased as the concentration was raised.

Putrescine was more effective on increasing fresh weight of Chrysanthemum plants, as compared with application of Uniconazole. This may be explained that Putrescine enhanced the accumulation of the photosynthetic products in the plant tissues, i.e. flowers.

Dry weight of inflorescence

Using Putrescine at 100, 200 and 300 ppm gave the values of 2.4, 3 .0 and 2.7 g as compared with the control 1.58 g in the 1st season. Similar trend was found in the 2nd season.

Application of Uniconazole at 20, 40, and 60 ppm recorded the values 1.94, 1.91 and 1.76 g for the 1st season. The same trend was found in the 2nd season. From these results it was noticed that using Putrescine at 200 ppm and Uniconazole at 20 ppm resulted in the highest increase in the dry weight of chrysanthemum flowers. These results are in agreement with those obtained by (Singh and Bist ,2003) on rose, (Mahgoub *et al.*, 2006 b) on *Dianthus caryophyllus* and (Mahgoub *et al*.,2006 a) on *Calendula officinalis*, (El –Quesni *et al.* ,2007) on *Bougainvillea glabra* plants, they found that paclobutrazole or Putrescine increased dry weight of flowers.

These results may be due to the promotive effect of Putrescine, which is essential for plant growth and differentiation and thus involved in various physiological processes (Flores and Galston, 1982 and Friedman *et al*., 1989).

Chemical constituents

a- Carbohydrate content

Data in Table (3) showed that all concentrations of the two bioregulators increased the total carbohydrates content in flowers of chrysanthemum and the highest values were found when the plants were treated with Putrescine at the

rate of 200 ppm and Uniconazole at the rate of 20 ppm. These increment in total carbohydrates content may be attributed to the increase in photosynthetic process efficiency, which led to increase assimilation of leaf CO_2.These results are in agreement with those obtained by, (Mahgoub *et al*.2006 b) on *Dianthus caryophyllus* and (El-Quesni *et al*., 2007) on *Bougainvillea glabra* plants as they obtained increases in the total carbohydrates content in the plants treated with different concentrations of Putrescine.

Concerning the effect of Uniconazole on the carbohydrates content in plants, these results are in agreement with those obtained by ,(Mahgoub *et al.*, 2006 a) on *Calendula officinalis* , (Bekheta *et al.*, 2003) on *Thymus serpyllum* plants and (El-Quesni *et al.*, 2007) on *Bougainvillea glabra* plants.

b. Photosynthetic pigments

Chlorophyll (a)

Data in Fig. (1) Illustrated that spraying Chrysanthemum plants with Putrescine significantly increased the content of Chl. (a) in recent leaves of plants.

In the 1st spray data showed that Treating plants with 200 ppm Putrescine caused the highest values of the content Chl. (a) for the 1st and 2nd seasons, respectively, followed by 300 ppm Putrescine, whereas the lowest concentration resulted in the lowest values of Chl. (a) for the control plants in the three sprays compared with the other treatments. The highest values were showed with plants treated with 200 ppm in the st spray, followed by the same concentration and in the 3rd spray at the two seasons, respectively.

Spraying the plants with 20 ppm significantly increased chl.(a) over the control in the 1st and 2nd season,respectively, followed by 40 and 60 ppm Uniconazole, which gave the lowest value of chl.(a) within the treatments but higher than the control in the two seasons (Fig.1).

In case of 2nd spray, in the 1st season, the data indicated that all the values of chl.(a) content were over control plants, when the plants were treated with 20, 40 and 60 ppm Uniconazole. The highest content was recorded in the plants treated with 20 ppm Uniconazole, which was higher than the untreated plants, whereas there was no different ratio, when the plants were treated with 40 and 60 ppm. In general chlorophyll (a) content clearly differed from control in the 1st spray of the two seasons, this means that the used bioregulators showed only their effect early at the beginning of their application, while after that the application was less effective.

Chlorophyll (b)

Spraying the plants with 200 ppm Putrescine produced the highest content of Chl. (b) in all sprays than that for control plants, while decreasing the concentration to 100 ppm reduced the content of Chl. (b) in both seasons (Fig. 2).

Regarding to Uniconazole treatments the data showed that spraying the plants with Uniconazole at the rates of 20, 40 and 60 ppm significantly increased the Chlorophyll (b) content; giving the highest content at the lowest concentration (20 ppm), while raising the concentration resulted in reduction of chlorophyll (b) content. The highest values were found in the 1st spray in 1st season.

The application of three sprays showed that their greatest effect had been obtained at the 1st spray.

Total carotenoids content

Data in Fig. (3) Revealed that, spraying Chrysanthemum plants with Putrescine and Uniconazole at the different concentrations affected significantly on increasing total carotenoids content in the two seasons. The highest values of carotenoids were obtained in plants treated with 200 ppm. Concerning Uniconazole treatment it was found that spraying the plants with 20 ppm resulted insignificant increase in the 1stand 2nd season at the 1st spray, followed by the 2ndand finally by the 3rd spray.

These results are in agreement with those obtained by (Talaat *et al*., 2005) on *Catharanthus roseus* and (Youssef *et al*., 2004) on *Matthiola incana*. Polyamines stimulated some physiological responses including vegetative growth and photosynthetic activity (Yanghua *et al*., 1996 and Chattopadayay *et al*., 2002). They found that different concentrations of Putrescine increased chl (a), (b) and total carotenoids, respectively.

With regard to the effect of Uniconazole on photosynthetic pigments, the obtained results are in harmony with those obtained by(Bekheta *et al*. ,2003) on *Thymus serpyllum* plants and(El-Quesni *et al*., 2007) on *Bouganivillea glabra* plants, they stated that treating the plants with triazole compound at 100 ppm concentrations increased chlorophylls contet.

Table 1. Flowering start after spraying of *Chrysanthemum indicum* L. plant as affected by Putrescine and Uniconazole during 2004/2005 and 2005/2006 seasons

	2004/2005				2005/2006			
Treatments	Dec.	Jan.	Feb.	Mar.	Dec.	Jan.	Feb.	Mar.
Control	4	11	18	15	8	14	17	15
Put., 100 ppm	15	22	28	20	17	24	26	20
Put., 200 ppm	25	28	35	26	24	29	33	28
Put., 300 ppm	27	31	22	25	29	33	24	22
Uni., 20 ppm	—	5	10	7	—	6	10	9
Uni., 40 ppm	—	7	6	9	—	9	5	9
Uni., 60 ppm	—	5	7	5	—	4	6	6

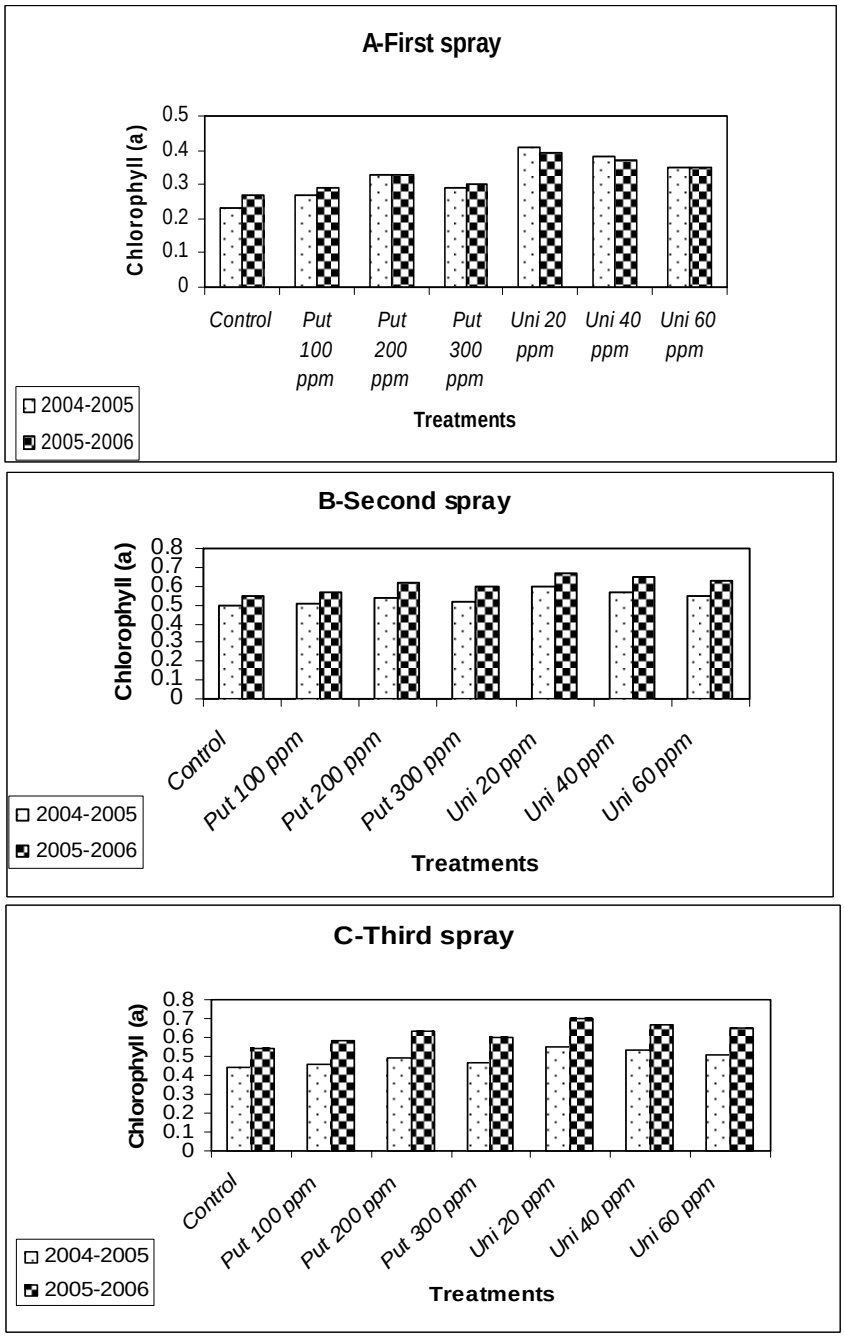
Table 2. Effect of Putrescine and Uniconazole on inflorescences characters of *Chrysanthemum indicum* L. plant during 2004/2005 and 2005/2006 seasons

Treatment	flower Yield /plant (No)		Flower diameter (cm)		Pedicle length (cm)		Length of inflorescence stalk (cm)		Vase life (days)		Fresh weight (g)		Dry weight (g)	
	Season													
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	72	078	5.6	5.9	8.9	9.0	20.7	22.0	11.3	14.3	4.5	4.6	1.58	1.60
Put., 100 ppm	104	108	7.5	7.7	9.1	9.8	27.3	28.6	20.7	22.0	6.3	6.6	2.40	2.45
Put., 200 ppm	147	161	8.0	8.6	10.0	11.5	30.6	32.3	26.0	27.0	7.1	8.2	2.99	3.12
Put., 300 ppm	108	120	7.8	8.1	9.8	10.1	28.2	29.8	24.3	24.0	7.0	7.3	2.66	2.59
Uni ., 20 ppm	103	104	6.8	7.2	7.5	08.7	18.1	19.2	19.0	20.0	5.3	6.3	1.94	2.30
Uni ., 40 ppm	097	100	6.9	6.5	6.8	07.7	17.8	18.7	18.0	18.3	5.3	6.0	1.91	2.18
Uni ., 60 ppm	089	090	6.6	6.4	6.0	07.0	16.6	17.3	17.0	18.0	4.9	5.0	1.76	1.78
L.S.D 0.05	4	5	1.1	0.9	1.4	0.9	2.2	2.4	2.0	2.4	0.2	1.1	0.15	0.54

Table 3. Carbohydrates contents (D.W %) in flowers of *Chrysanthemum indicum* L. plant as affected by Putrescine and Uniconazole treatments during 2004/2005 and 2005/2006 seasons.

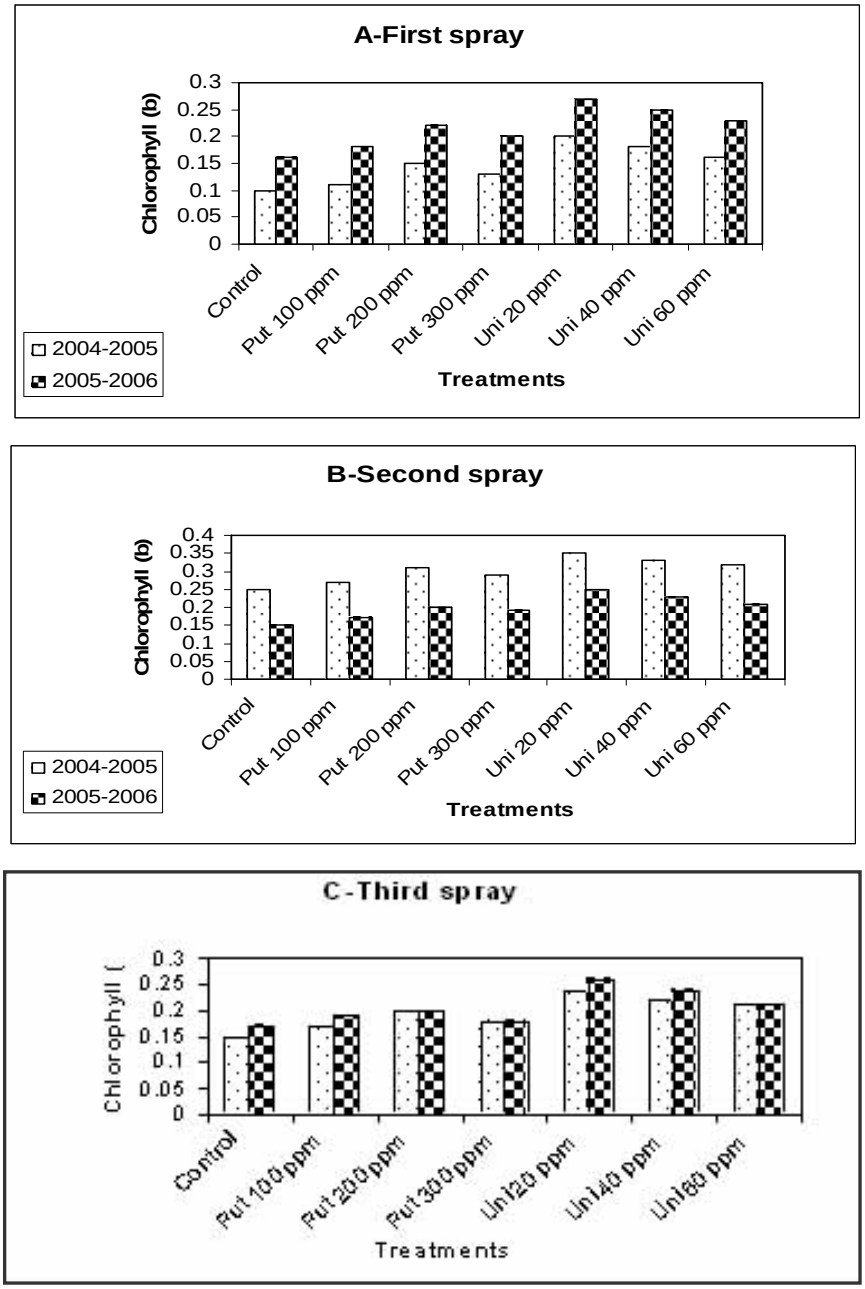
Treatment	Flowers	
	2004/2005	2005/2006
Control	10.8	14.3
Put., 100 ppm	15.0	14.3
Put., 200 ppm	17.4	21.7
Put., 300 ppm	15.0	19.6
Uni., 20 ppm	19.7	21.5
Uni., 40 ppm	16.8	16.0
Uni., 60 ppm	14.0	15.8
L.S.D 0.05	3.7	2.1

Put= Putrescine Uni= Uniconazole



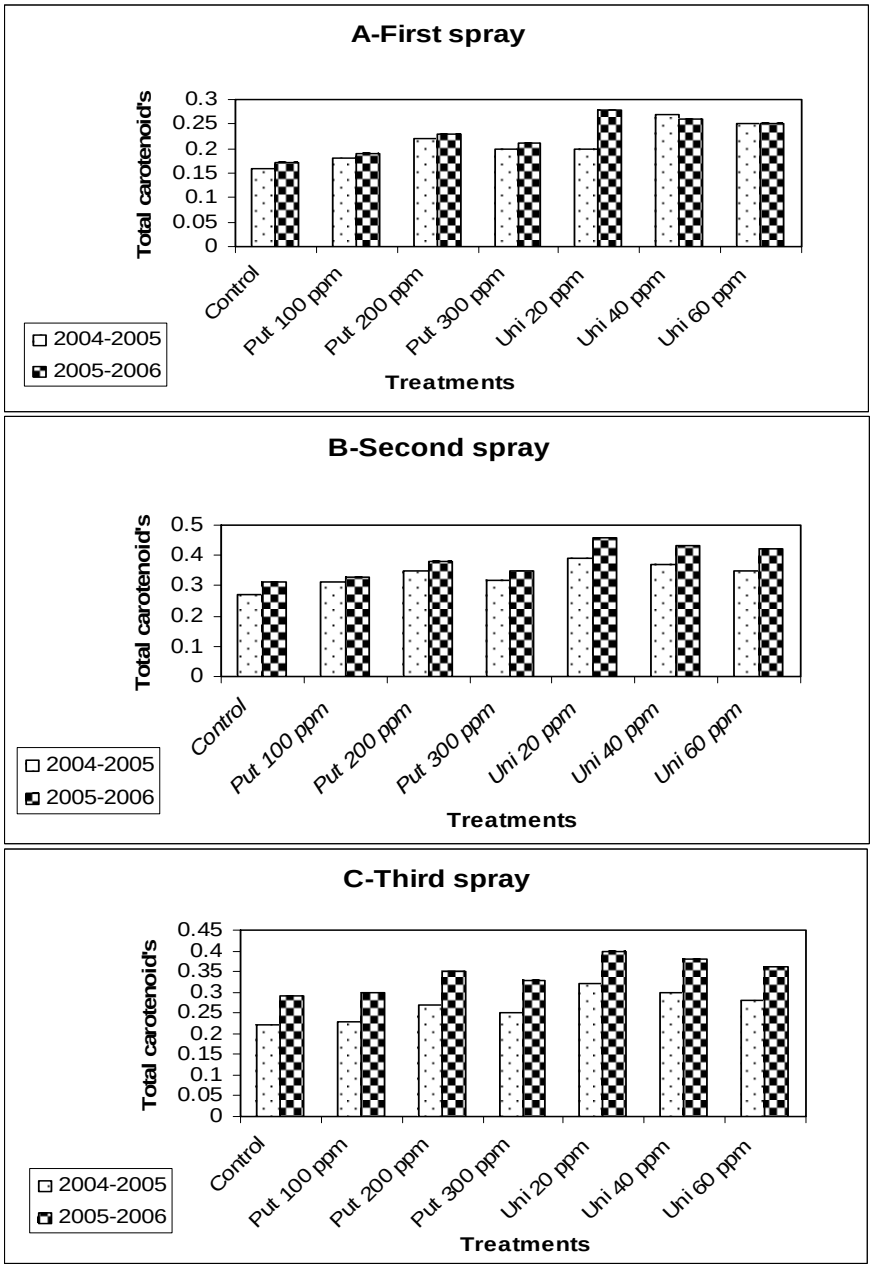
Put= Putrescine Uni= Uniconazole

Fig.1. Chlorophyll (a) content (mg/g F.W) of *Chrysanthemum indicum* L.plant as affected by Putrescine and Uniconazole treatments during 2004/2005 and 2005/2006 seasons.



Put= Putrescine Uni= Uniconazole

Fig. 2. Chlorophyll (b) content (mg/g F.W) of *Chrysanthemum indicum* L. plant as affected by Putrescine and Uniconazole treatments 2004/2005 and 2005/2006 seasons.



Put= Putrescine Uni= Uniconazole

Fig . 3. Total carotenoids content (mg/g F.W) of *Chrysanthemum indicum* L. plant as affected by Putrescine and Uniconazole treatments 2004/2005 and 2005/ 2006 seasons.

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