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INFLUENCE OF BIOREGULATORS UNICONAZOLE AND COUMARIN ON SOME MORPHOLOGICAL, PHYSIOLOGICAL ASPECTS AND FIBER QUALITY OF COTTON PLANTS

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ABSTRACT

Two field experiments were carried out in two successive seasons (2001 and 2002) at the middle of Delta, El-Mahalla El-Kobra region, Gharbia Governorate to study the physiological responses of cotton plants to bioregulators uniconazole at 20, 40 and 60 ppm or coumarin at 50, 100 and 200 ppm. This work aimed at studying the effect of these bioregulators on vegetative growth parameters, some biochemical constituents (e.g. endogenous hormones, photosynthetic pigments, oil and fatty acids composition of the produced seeds) and the reflection of all these physiological changes on the yield and fiber properties of cotton (*Gossypium barbadense*, Cv. Giza 86) plants.

The obtained results revealed that plant height, internode length and the number of internodes/plant were significantly decreased mostly at all the used levels of bioregulators. Meanwhile, number of leaves/plant and the number of branches/plant were significantly increased at 40 or 60 ppm of uniconazole and at 100 or 200 ppm of coumarin. Application of uniconazole at the rate of 40 or 60 ppm and coumarin at the rate of 100 or 200 ppm led to mostly significant increases in number of flowers/plant, number of bolls/plant, boll setting %, number of open bolls/plant, seed cotton yield/plant and seed cotton yield/fed. Also, the fiber properties, e.g. uniformity, fiber length, fiber strength, elongation and micronaire reading of the produced cotton, were improved and increased mostly at all used levels.

The application of bioregulators at all the used levels led to decreases in the content of endogenous hormones indole acetic acid (IAA) and gibberellic acid (GA_3), but the endogenous content of abscisic acid (ABA) was increased. While the photosynthetic pigments (chl. a, chl. b and carotenoids) were significantly increased mostly at all the used levels of uniconazole. Oil percentage and fatty acid composition of the produced seeds were improved mostly at all the used levels of uniconazole and coumarin.

Keywords: uniconazole, coumarin, endogenous hormones, fatty acids, photosynthetic pigments, fiber properties, cotton.

INTRODUCTION

Cotton plants (*Gossypium barbadense*) is a major economic field crop in the world as well as in Egypt. It is very responsive to environmental changes. The shade within the plant canopy increases the vegetative growth and consequently increases fruit abscission which in turn leads to reduction in yield production (Guinn, 1974). Several investigators are interested in the use of growth regulators especially growth retardants in order to control the undesirable increment in vegetative growth and improving cotton yield (Zhang *et al.*, 1990; Oosterhuis and Egilla, 1996; Reddy *et al.*, 1996; Abdel-Aal, 1997; Crozat *et al.*, 1997; Ghourab *et al.*, 2000; Prasad *et al.*, 2000 and Zhao and Oosterhuis, 2000).

Several triazole compounds are known to be effective inhibitors of

elongation and improve the yield which is the main purpose of using it (Wample and Culver, 1983; Sankhela *et al.*,1985; Rademacher *et al.*, 1987; Wood, 1988; and Fouda and Ramadan, 2000). In this concern, Khalil and Al-Abdulkreem (2002) on wheat plant found that application of different concentrations of uniconazole resulted in significant decrease in plant height which was accompanied by significant increase in the total number of tillers, leaves and branches and reflected positively on the yield production (number of spikes, spike weight, grain weight/plant straw weight/plant and weight of 100-grains). El-Kady (2002) stated that the use of uniconazole increased the photosynthetic pigments in wheat plants.

Coumarin and its derivatives are naturally synthesized by the plants of all families (Murray *et al.*, 1982) and now ones are identified every year (Murray, 1991). Coumarin is acting as plant growth regulators which affect many plant activities (Zobel and March, 1993). In this connection, Veverka (1992) reported that coumarins and paclobutrazol reduced plant height of *Medicago sativa* to 65 and 86%, respectively and also increased the number of racemes/100 stems and 100-seeds weight. Ohira and Yatagi (1994) reported that Coumarin had an inhibitory effect on stem elongation of different plants (e.g. radish, lettuce, alfalfa, turnip and mung bean). Moreover, Ramadan (1998) indicated that foliar application or soaking the seeds *of Vicia faba* in different concentrations of coumarin decreased the vegetative growth parameters (plant height, number of leaves and nodes, leaf area, fresh and dry weights of shoot system) and also found that coumarin treatments decreased the photosynthetic pigments (chl. a & chl. b and carotenoids). Also, Mohsen and Kulkuttawi (1989) found that coumarin treatments were slightly suppressive to pigments accumulation after prolonged soaking (24hr), only during the early part of the experiment in tomato seeds and there is a fluctuation in pigments content of cabbage seedlings revealing that during the 1st part of the experiment, the short term soaking of coumarin remarkably enhanced chl. a & chl. b accumulation.

The used growth retardants enhances seed's oil content of different treated plants (Khafaga, 1986 on fennel plants; Mekki and El-Kholy, 1999 on rape plants and Sawan *et al.*, 2001 on cotton plants) they all found that application of growth retardants at different levels led to increases in the oil yield/ha and seed oil refractive index. The fatty acids composition is also influences by the application of growth retardants e.g. pix (El–Shahaby *et al.*, 1994; Abdel-Rehim *et al.*, 2000).

All triazoles compounds, its derivatives (uniconazole, paclobutrazol etc....) and coumarin act as antigibberellin i.e. interfere with the biosynthesis of endogenous gibberellin by preventing the oxidation of entkauren to entkauronic acid (Izumi *et al.*, 1988; Rademacher *et al.*, 1987; Bekheta 2000 and Bekheta *et al.*, 2003). These compounds also reduced the endogenous content of auxins (IAA) but led to increase of the endogenous content of abscisic acid (ABA) in treated plants (Taniya *et al.*, 1986; Hassanein *et al.*, 1987; Ramadan, 1998; Bekheta, 2000 and Bekheta *et al.*, 2003).

This work aimed to study the effect of growth retardants (uniconazole or coumarin) on vegetative growth parameters, some biochemical constituents (e.g. endogenous hormones, photosynthetic pigments, oil and

fatty acids composition of the produced seeds) and the reflection of all these physiological changes on the yield and fiber properties of cotton plants.

MATERIALS AND METHODS

Two field experiments were carried out at the middle of Delta, El-Mahalla El-Kobra region, Gharbia Governorate. Seeds of cotton (*Gossypium barbadence*, cv. Giza "86") were planted at 22 of March during the two successive seasons 2001 and 2002. Thining was practiced at 30 days after sowing. Phosphorus fertilizer was added at the rate of 22.5 kg P_2O_2/fed. as calcium superphosphate (15.5% P_2O_2) during land preparation and divided into two equal portions, the first portion was added after thinning and the second portion was applied at the next irrigation. The other cultural practices were carried out as recommended for the conventional cotton planting.

The plants were sprayed twice (at 70 and 84 days after sowing) with freshly prepared solutions of uniconazole at the rate of 20, 40 or 60 ppm and coumarin at the rate of 50, 100 or 200 ppm while the control plants were sprayed with tape water. The volume of the spraying solution was maintained to cover completely the plant foliage till drip.

Plant samples were collected before flowering and were used for the following chemical estimations: photosynthetic pigments, endogenous hormones (auxins, abscisic acid and gibberellic acid). Other plant samples were collected at harvest and the following parameters were recorded: plant height (cm), internode length (cm), leaf area (cm^2) number of nodes/plant, number of leaves, number of branches/plant, flowers and bolls/plant, boll setting % of open bolls, seed cotton yield/plant (g) and seed cotton yield/fed (kentar). The produced seeds were collected from each treatment and used for the estimation of fatty acids and oil seed percentage while the lint cotton was used for determination of fiber and yarn properties.

Biochemical analysis:

Photosynthetic pigments:

Photosynthetic pigments (Chlorophyll a, b and carotenoides) were determined according to the spectrophotometric method as recommended by Metzner *et al.* (1965).

Endogenous hormones:

Extraction, purification and separation of plant hormones were carried out according to Shindy and Smith (1975). Methylation of plant hormones with diazo methane was carried out according to Vogel (1975) and estimation was done by Gas Liquid Chromatography (GLC).

Seed oil content:

The method adopted for extraction and determination of the seed oil content was that described by Meara (1957).

Identification, fractionation, and quantification of fatty acids:

The fatty acids were converted to methyl esters according to Sink *et al.* (1964) method. The fatty acids methyl esters were injected into Gas Liquid Chromatography (6.0 feet x 1/8 inch internal diameter column packed with 20% diethylene glycol succinate on chromosorb 60-80 inch).

Fiber properties of cotton:

Fibers and yarn properties were determined according to ASTM method (A.S.T.M, D- 1440-67) for the fiber length by fiber graph 630 and (A.S.T.M., D-1448-75, 1984) for fiber strength by Stelometer and also Micronaire reading of cotton fiber measured by Micronaire (A.S.T.M, D–1448-59, 1984).The single yarn strength and elongation percentage were determined on Zwick 1511 Automatic Tensile Tester (A.S.T.M., D-2256-84).

Experimental design was randomized complete blocks with four replications and the combined analysis was made for the two growing seasons due to the similarity of the results in both seasons according to Snedecor and Cochran (1980). The L.S.D test was used to evaluate treatment means.

RESULTS AND DISCUSSION

Vegetative growth

Perusal of the data in Table (1) showed that foliar application of both uniconazole and coumarin on cotton plants before blooming stage significantly decreased plant height, internode length and leaf area. The magnitude of reduction was related to the highest concentration of each growth regulator 60 and 200 ppm for uniconazole and coumarin, respectively. Also, the number of nodes/plant was not significantly decreased in response to 20 & 40 ppm uniconazole and 50 and 100 ppm coumarin, but using uniconazole at 60 ppm led to significant decrease. The reduction in plant height was due to reduction in internode length in treated plants (Lecain *et al.*, 1986). These findings were supported by many investigators (Wample and Culver, 1983; Lecain *et al.*, 1986; Veverka, 1992; Al-Abdulkreem, 1993). Moreover, Lethi and Bilayalieva (1983); Ohira and Yatagi (1994) and Ramadan (1998) showed that coumarin had an inhibitory effect on plant height of different plants, the remarkable inhibition of plant height may be attributed to the effect of these growth retardants on gibberellins biosynthesis which are antigibberellin, i.e. prevent the conversion of kaurene to kaurenoic acid which leads to the formation of gibberellin (Hedden and Graebe, 1985). In addition, these growth retardants tend to reduce the synthesis and action of auxins in plants through enhancing the activity of IAA-oxidase as well as reducing the rate of transformation of trypotophan to IAA (Wang *et al.*, 1998).

Number of leaves/plant and the number of branches/plant were mostly increased significantly due to the application of both uniconazole and coumarin at all used concentrations. In this respect, Bekheta (2000) on *Vicia faba*; EL-Kady (2002) on wheat and Khalil and Al-AbdulKreem (2002) on wheat reported that using uniconazole caused a significant increase in the numbers of both leaves and branches/plant. The findings of the present study are in agreement with those of Braun and Garth (1986) on strawberry; Maus (1987) on Hibiscus rose; Bekheta *et al.* (2003) on *Thymus serpyllum* and Bekheta (2004) on wheat plant. Meanwhile, the effect of coumarin on the aforementioned growth parameters was also confirmed by Gupta (1990) who found that application of coumarin at the rate of 50, 100 or 200 ppm increased number of leaves, branches, nodes as well as fresh and dry weights of *Vicia faba* shoots. The increase in the number of branches of

cotton plants as a result of foliar application of both growth retardants may be attributed to the high level of cytokinins, accompanied by reduced levels of indole acetic acid and gibberellins which lead to inhibition of main stem apical dominance (Al- Abdulkreem, 1993 and Ramadan, 1998).

Table (1): Effect of preblooming foliar spray of uniconazole and coumarin on vegetative growth parameters of cotton plants.

Characters / Treatments	Plant Height (cm)	Internode Length (cm)	No. of nodes/ plant	Leaf area (cm^2)	No. of leaves/ plant	No. of branches /plant
Control	110.00	4.26	25.00	11.00	25.00	5.00
Uniconazole 20 ppm	105.00	4.10	24.80	10.69	29.00	5.50
40 ppm.	90.50	3.10	24.00	9.56	36.00	9.00
60 ppm	80.40	3.00	23.80	9.05	33.00	7.00
Coumarin 50 ppm	103.00	4.30	25.00	11.20	25.50	5.00
100 ppm	104.00	4.29	26.20	9.32	30.00	6.80
200 ppm	100.00	4.00	26.00	9.00	32.00	7.80
L.S.D (5%)	5.85	0.31	1.24	0.75	7.00	0.82

Yield and its components:

Data presented in Table (2) showed that the relatively higher concentration of uniconazole (40 or 60 ppm) and coumarin (100 or 200 ppm) resulted in significant increases in yield parameters of cotton plants (number of flowers, number of bolls, boll setting % and number of open bolls) which were accompanied by significant increases in seed cotton yield/plant (g) and seed cotton yield/fed. The magnitude of increments was more pronounced with 40 ppm uniconazole and 100 ppm coumarin concerning all yield parameters. Meanwhile, the low concentration of 20 ppm uniconazole and 50 ppm of coumarin did not significantly affect all measurements of yield. In this connection, numerous studies on cotton plants found that foliar application of growth retardant (pix) increased the number of sympodia, percentage of flowers and bolls retention, earliness, seed index as well as seed cotton yield (Reddy *et al.*, 1996; Crozat *et al.*, 1997; Nepomuceno *et al.*, 1997; Oosterhuis *et al.*, 1998; Mekki, 1999; Ghourab *et al.*, 2000 and Prasad *et al.*, 2000).

Table (2): Effect of preblooming foliar spray of uniconazole and coumarin on number of flowers, yield and its components of cotton plants.

Characters / Treatments	No. of flowers /plant	No. of bolls/ plant	Boll setting %	No. of open bolls/ plant	Seed cotton yield/ plant (g)	Seed cotton yield/ fed. (kantar)
Control	25.70	15.00	58.72	10.13	35.13	7.16
Uniconazole 20 ppm	24.50	14.50	59.18	10.00	34.00	6.93
40 ppm.	35.00	24.50	70.00	14.80	43.95	8.96
60 ppm	30.00	21.00	70.00	12.90	37.50	7.64
Coumarin 50 ppm	24.50	21.00	66.67	11.10	35.00	7.13
100 ppm	28.00	18.90	67.14	11.90	37.10	7.56
200 ppm	29.50	18.19	61.66	12.00	36.00	7.34
L.S.D (5%)	2.91	1.60	5.18	0.72	2.44	0.83

The increase in yield parameters due to uniconazole application was also confirmed on wheat plants by Khalil and Al-Abdulkreem (2002) and Bekheta (2004), while the use of coumarin was confirmed by Gupta (1990) on *Vicia faba*; Veverka (1992) on *Medicago sativa* and Ramadan (1998) on *Vicia faba*. The improvement of cotton yield due to application of growth retardants may be attributed to their effect on the content of photosynthetic pigments and consequently the increase of assimilates which are directed to fruit filling rather than stem elongation or vegetative growth (Treharne *et al.*, 1985 and Jung *et al.*, 1987).

Fiber properties:

The presented data in Table (3) showed that foliar application of uniconazole at the rate of 40 or 60 ppm and coumarin at the rate of 50 or 100 ppm on cotton plants at preblooming stage led to significant increases in all fiber properties (fiber length, fiber strength, elongation% and micronaire reading). The maximum values were obtained from the application of higher concentrations 60 & 100 ppm of uniconazole and coumarin, respectively. On the other hand, all lint properties were not significantly affected by using the lowest concentrations (20 and 50 ppm) of uniconazole and coumarin, respectively. These results are in conformity with Abdel-Al and Eid, 1985; Eid *et al.*, 1986; Cathey and Meredith, 1988; Ebelhar *et al.*, 1996; Lege *et al.*, 1996 and Mekki, 1999) who found that foliar application of growth retardant pix increased significantly fiber length (2.5% and 12.5% span length), fiber elongation% reached to 15.89%, maturity ratio, micronaire reading and Yarn strength of cotton plants.

Table (3): Effect of preblooming foliar spray of uniconazole and coumarin on fiber properties of cotton plants.

Properties / Treatments	Uniformity (%)	Fiber length (mm)	Fiber strength (g/tex)	Elongation (%)	Micronaire reading
Control	86.10	32.80	38.00	6.90	4.50
Uniconazole 20 ppm	86.60	32.20	37.00	6.90	4.40
40 ppm.	88.90	34.00	42.40	7.40	4.60
60 ppm	88.70	35.00	43.00	7.10	4.80
Coumarin 50 ppm	87.80	34.20	37.00	7.00	4.80
100 ppm	89.20	34.20	40.30	7.50	4.90
200 ppm	86.50	33.20	38.40	7.30	4.60
L.S.D (5%)	N.S.	1.37	3.30	0.51	0.20

Biochemical analysis:

Photosynthetic pigments:

Perusal of the data in Table (4) revealed that foliar application of uniconazole at preblooming stage on cotton plants exhibited significant increases in all photosynthetic pigments (chl. a, chl. b, chl. a+b and total carotenoides) in response to 40 and 60 ppm and non significant increases in response to 20 ppm for chl. b and carotenoides. These results are in agreement with those obtained by several investigators (Sankhela *et al.*, 1985 on soybean; Fletcher and Hofstra 1990; on wheat; Wang *et al.*, 1995; Bekheta, 2000 on *Vicia faba*; El-Kady, 2002 on wheat; and Bekheta *et al.*,

2003 *on Thymus serpyllum*) who showed that uniconazole treated plants typically appeared darker green color than untreated once due to the increase in photosynthetic pigments. This increment may be attributed to the effect of uniconazole on the enhancement of cytokinins level and their role on chlorophyll's biosynthesis (Fletcher and Arnold, 1986). In addition, cytokinins has a role in increasing photochlorophyllide content and increasing the activity of chlorophyll synthetase (Chen, 1990).

Table (4): Effect of foliar spray with uniconazole or coumarin on photosynthetic pigments (mg/100 gm fresh weight) of cotton plants.

Pigments / Treatments	Chl. A	Chl. b	Chl. a + b	Carotenoids
Control	210.12	115.07	325.19	48.20
Uniconazole 20 ppm	255.10	123.21	378.31	51.67
40 ppm.	287.20	166.10	453.30	65.46
60 ppm	288.37	183.75	472.12	67.01
Coumarin 50 ppm	211.05	115.09	326.14	49.00
100 ppm	207.05	108.80	315.85	51.07
200 ppm	201.11	102.09	303.20	53.00
L.S.D (5%)	22.61	10.78	30.67	4.96

Regarding the effect of coumarin treatments at all photosynthetic pigments, the results in Table (4) showed a non-significant increases with all used concentrations. These results agree with Knypl (1971) who indicated that coumarin and some plant growth retarding inhibited chlorophyll synthesis as a consequence of protein synthesis inhibition Also, Mohsen and Kulkuttawi (1989) recorded that pretreatment with coumarin at the rate of 10-3M slightly reduced the pigments accumulation after prolonged soaking (24 hr only) during early part of the experiment in tomato plants.

Endogenous phytohormones:

Data in Table (5) indicated that foliar application of uniconazole or coumarin at all levels, in the preblooming stage, led to significant decrease in the content of endogenous phytohormones (IAA & GA_3). On the other hand, the content of ABA was increased gradually due to the application of both growth retardants. Similar results using triazole compounds were previously recorded by Bekheta (2000) on *Vicia faba*; Fouda and Ramadan (2000) on maize; and Bekheta *et al.* (2003) on *Thymus serpyllum*. The coumarin results obtained in this work are in agreement with those obtained by Taniya *et al.* (1986) on *Phaseolus vulgaris*; Hassanein *et al.* (1987) on sorghum and Ramadan (1998) on *Vicia faba*.

The decrease in the endogenous content of IAA caused by the application of uniconazole or coumarin might be attributed to the stimulating activity of IAA oxidase which in turn led to inhibition in the rate of transformation of trypotophan to IAA (Wang *et al.*, 1998). This enzyme is known to control the auxins level in plants (Reinecke and Bandurski, 1988) The decrease in the endogenous content of GA_3 resulted from using these growth retardants are in agreement with those obtained by several investigators (Hedden and Graebe, 1985; Izumi *et al.*, 1985; Lenton *et al.*, 1987; Rademacher *et al.*, 1987; El-Kady, 2002 and Bekheta *et al.*, 2003).

Table (5): Effect of preblooming foliar spray of uniconazole or coumarin on the endogenous hormones (µg/gm fresh weight) of cotton plants.

Hormones / Treatments	IAA	GA_3	ABA
Control	11.211	95.780	4.864
Uniconazole 20 ppm	6.893	71.530	5.519
40 ppm.	5.083	52.170	5.990
60 ppm	3.015	39.280	6.130
Coumarin 50 ppm	6.193	73.290	4.930
100 ppm	6.936	71.050	5.340
200 ppm	5.308	68.820	7.370

Oil seed percentage:

It is clear in Table (6) that cotton plants sprayed with uniconazole or coumarin exhibited significant increases in the percentage of cotton seed oil. The maximum values were recorded in response to 40 ppm uniconazole or 100 ppm coumarin as compared with those obtained from untreated plants. Thus, it can be concluded that the oil contents of cotton seeds were differently affected through the response of hydrolytic activity of oil to the different levels of growth retardants. This conclusion was in accordance with Shive and Sisler (1976) who reported that the increase in the essential oil content in response to pix was due to their capability of preventing the hydrolytic breakdown of oil.

Table (6): Oil percentage of cotton seeds as affected by preblooming spray with uniconazole or coumarin.

Treatments / Characters	Control	Uniconazole (ppm)			Coumarin (ppm)			L.S.D (5%)
		20	40	60	50	100	200	
Seed oil (%)	18.58	20.01	20.62	17.29	19.27	21.78	20.47	1.09

Fatty acids composition:

As regard to Table (7), the results showed that all used levels of uniconazole mostly caused a marked increase in the saturated and unsaturated fatty acids, especially linoleic acid which constitute a predominant unsaturated fatty acid in cotton seed oil, except for linolenic acid which showed a slight decrease in response to 20 or 40 ppm and marked decrease in oleic acid in response to 60 ppm of uniconazole.

Concerning the effect of coumarin, the relatively low concentrations (50 or 100 ppm) caused a remarkable increase in all saturated and unsaturated fatty acids, except for Heptadcanoic, oleic and linoleic which showed a marked decease in cotton seeds oil. However, the highest concentration of coumarin (200 ppm) in general, induced a slight reduction in the saturated and unsaturated fatty acids. The total saturated fatty acids of cotton seeds oil were increased at all used levels of uniconazole and coumarin. The maximum value of total saturated fatty acids was obtained from the application of coumarin at 100 ppm and uniconazole at 40 ppm, being 42.19 and 33.80%, respectively. The enhancement of total saturated

Table (7): Fatty acids composition of cotton seeds oil as affected by preblooming foliar spray with uniconazole or coumarin.

Treatments / Fatty acids (%)	Control	Uniconazole (ppm)			Coumarin (ppm)		
		20	40	60	50	100	200
Lauric acid (C_{12})	0.86	1.01	0.97	0.96	0.86	0.98	0.86
Palmetic ($C_{16:0}$)	25.69	28.91	27.56	26.91	28.08	30.50	25.50
Heptadecanoic ($C_{17:1}$)	0.21	0.23	0.20	0.20	0.20	0.22	0.26
Olieic A. ($C_{18:1}$)	18.02	20.64	19.50	5.60	15.01	16.92	18.40
Linoleic ($C_{18:2}$)	27.69	36.69	40.38	30.38	22.20	26.20	27.52
Linolenic ($C_{18:3}$)	0.17	0.13	0.11	7.60	0.43	0.37	0.38
Arachidic ($C_{20:2}$)	0.50	0.54	0.51	0.53	1.65	1.94	0.99
Eicosadieonic ($C_{20:2}$)	2.14	2.30	2.92	9.50	2.30	3.85	2.07
Erucic A ($C_{22:1}$)	0.30	0.38	0.30	3.23	0.33	0.42	0.26
Tricosanoic ($C_{23:0}$)	0.76	1.02	3.99	3.19	5.93	7.69	4.94
Lignoceric ($C_{24:0}$)	1.63	2.12	0.77	0.57	1.00	1.08	0.87
Saturated	29.44	33.60	33.80	32.16	37.52	42.19	33.16
Unsaturated	48.53	60.37	63.41	56.51	40.47	47.98	48.89
Unsaturated/Saturated	1.65	1.80	1.88	1.76	1.08	1.14	1.48

fatty acids at all used levels of both growth retardants were accompanied by increments in the total unsaturated fatty acids with using uniconazole and reduction with using coumarin. The high content of total unsaturated fatty acids in the oil of the uniconazole treated plants led to increase in the rate of total unsaturated/saturated fatty acids. It means that the oil was a good source of essential fatty acids for human food. Thus, it could be concluded that uniconazole is better than coumarin in improvement of the oil quality. In this connection, Abdel-Rehim *et al.* (2000) indicated that alar caused an increase in the percentage of capric, lauric, myristolic, stearic, palmatic, behanoic and oleic acids, while the percentage of lenoleic acid was significantly deceased in datura seed oil. Sawan *et al.* (2001) indicated that foliar application of growth retardants (cycocel, pix and alar) to cotton plants increased unsabonifable matter and total unsaturated fatty acids (oleic and linolenic acids) and decreased sabonifable value and total saturated fatty acids in cotton seed oil.

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therionine (amino acid contains S-group), alanine, serine and valine (aliphatic amino acids) concentrations in tall Fescue plant occurred under low soil water availability , compared with adequate water conditions.

Many plants and other organisms cope with osmotic stress by synthesizing and accumulating some compatible solutes, which are termed as osmoprotectants or osmolytes. These compounds are small, electrically neutral molecules, which are non-toxic even at molar concentrations (Alonso *et al.*, 2001). During osmotic stress, plant cells accumulate solutes to prevent water loss and to re-establish cell turgor. The solutes that accumulate during the osmotic adjustment include ions such as K^+,Na^+, and Cl^- or organic solutes that include nitrogen containing compounds, such as proline and other amino acids, polyamines and quaternary ammonium compounds (Tamura *et al.*,2003 and Reddy *et al.*, 2004)

Drought tolerant plants can use several mechanisms to adapt water stress. These include reduction in water loss by increased stomatal resistance or increased water uptake by the development of large and deep root systems, increase in cyclic amino acid (Dubey, 1994 and Nour El-Din 1995).

The study of plant responses to water stress has been studied by physiologists who attempts to understand how plants function in their natural environment (Osmond *et al.*, 1987). Plants either avoid or tolerate periods of drought, often accompanied by high temperature and excessive irradiance levels (Ehleringer and Cooper, 1992), through phonological, morphological and physiological adjustments (Turner, 1986 and Morsy 2002).

Halophytes are plants, which are able to live under elevated salinities in their growth media, the salinity level in which they grow varies from slight, brackish medium to severe and may reach levels above seawater salinity (Gallagher, 1985 and Zahran, 1982).

The present study aimed to elucidate some physiological adaptive responses of species under the influence of different habitat conditions. For this reason, protein accumulation or concentration, protein amino acid and protein electrophoresis in the studied species were carried out to identify various protective adaptive responses of species growing in drought and saline stresses.

MATERIALS AND METHODS

The plant materials were collected from the various habitat of Wadi EL-Gafra in mid-July 2003. The habitat types were as follows:

I. Xeric habitat

Included two xeric plants represented by *Panicum turgidum*, Forssk and *Lasiurus hirsutus*, Forssk. These plants were collected from ups tream portion of Wadi El-Gafra, near the high way at 63km west of Suez, Egypt.

II. Saline habitats

Involved both of *Tamarix mannifera*, Ehrenb. and *Nitraria retusa*, Forssk. Collected from Midstream portion of Wadi EL-Gafra. Just N-NW EL-Asher Min Ramadan City, Egypt.

1. Chemical Analysis:

1.1. Total crude protein%:

Determination of total nitrogen and total protein as expressed by mg/g dry wt. were estimated using kjeldahl method (James, 1995).

1.2. Protein Amino Acids:

The hydrolyzed protein amino acids were determined according to the method described by Pellet and Young (1980).Defatted plant powder was dissolved in 6N HCl in a sealing tube. The mixture was hydrolyzed at 110°C for 24 hours; then the hydrolyzate was dried under vacuum at 70°C, and dissolved in sodium citrate buffer(pH2), and injected in the Amino Acid Analyzer.

1.3. Protein electrophoresis:

S.D.S polyacrylamide gel electrophoresis (S.D.S. PAGE) was performed for total proteins of the four different species according to the method of Laemmli (1970), as modified by Studier (1973).

RESULTS AND DISCUSSION

1.1.2. Crude Protein and Protein Amino Acids:

Protein synthesis is closely related to production of new tissue, which is the principle sink for nitrogenous compounds, and it is not surprising that when water stress inhibits growth, nitrogen metabolism is disturbed. An overall decrease in the levels of total protein content in plants growing under water stress conditions compared with plants growing under non-stressed environments was documented (Ramanjulu and Suduhakar, 1995).

The results in Table (1) show that the total crude protein and protein amino acids concentrations of different plant species in two different habitat conditions. The data indicate variation among the different plant species and locations in amino acid numbers and concentrations.

The studied xerophytes and halophytes show generally marked interspecific variations in their building units of proteins. Each species has its own collective assortment and concentration of amino acids that varied in some respect or with other species.

Table(1) indicates that total protein content in *Nitraria retusa*(halophytic plants) reached to the maximum values (113.1 mg/g dry wt.),while reached to its minimum values (57.5 mg/g dry wt.) in *Lasiurus hirsutus* (xerophytic plant).

According to Bewely and Larson (1980), and Bewley (1981), the production or availability of substances (e.g. sugar, anions, amino acids including proline) to maintain bound water could be important features of desiccation tolerance.Very mild to moderate water-stress reduces the level of protein synthesis in drought sensitive vegetative tissue and protein synthesis does not recover in cells subjected to severe water loss.

The *Tamarix mannifera* accumulated the highest concentration of cyclic amino acids (Proline, Phenylalanine and Tyrosine) followed by *Nitraria retusa* (17.68 and 16.97mg/g d.wt), respectively.The role of proline in plants exposed to salt stress has been investigated by many authors (Soltani & Bernard, 1977 and Raper & Kramer, 1983).

Table (1). Total crude protein and protein amino acid contents of some different plants species which were collected from up and mid stream portion of wadi EL-Gafra.

Measurements		*Panicum turgidum*	*Lasiurus hirsutus*	*Tamarix mannifera*	*Nitraria retusa*
Total crude protein mg/g d.wt		78.1	57.5	107.5	113.1
Amino acid (mg/g d.wt)					
Acidic amino acid	Aspartic	14.5	7.30	7.11	6.83
	Glutamic	3.11	3.73	4.75	6.46
Basic amino acid	Lysine	10.5	3.10	4.02	3.98
	Histadine	2.42	2.24	3.36	3.25
	Arginine	------	2.30	3.10	2.90
Cyclic amino acid	Proline	8.42	10.3	12.3	12.1
	Phenylalanine	2.24	2.50	3.62	3.38
	Tyrosine	2.10	1.12	1.73	1.49
Amino acid contain S – group	Methionine	0.76	0.68	0.69	0.75
Aliphatic amino acid	Glycine	2.91	3.22	4.60	4.31
	Alanine	3.42	2.52	3.84	3.81
	Valine	2.91	2.66	4.55	4.28
	Leucine	4.23	2.11	5.56	5.25
	Isoleucine	1.63	2.20	3.29	3.09
	Therionine	2.11	2.51	3.50	3.30
	Serine	2.22	2.85	3.94	3.69
	Amino butaric acid	4.02	4.81	6.10	6.01
Total number of amino acid		16	17	17	17
Total concentrations of amino acid		67.43	56.15	76.09	74.88

Proline increased in both the halophytes *Tamarix tetraghne* and the glycophyte *Pisum sativum*, when grown at various levels of Na Cl, and thus considered as evidence that it may act as a cytoplasmic osmoticum. Also, it is the most stable amino acid resisting oxidative acid hydrolysis to toxins, and is the least inhibitory of cell growth among all amino acids. The bound water would increase due to the highly hygroscopic nature of proline (Reddy *et al.*,2004)

Also proline is known to be involved in reducing the photodamage in the thylakoid membranes by scavenging and /or reducing the production of $^1O_2$. Proline accumulation in plants is caused, not only by the activation of proline biosynthesis, but also by the inactivation of proline degradation, thereby resulting in a decrease in the level of accumulated proline in rehydrated plants. Proline degradation to glutamic acid via pyrroline-5-carboxylate in higher plants is catalyzed by proline dehydrogenase.(Reddy *et al.*,2004) .It can also be inferred that proline acts as a free radical scavenger and may be more important in overcoming stress than in acting as a simple osmolyte (Bohnert and Jenson,1996 ;Alonso *et al.*,2001; Pinhero *et al.*, 2001 and Tamura et al.,2003).

Results from Table (1) revealed that the increased of both glycine, serin and glutamic amino acids in plants grown in saline habitats became more accumulated than xeric habitats, were recorded 6.46 mg/g dry wt. in

Nitraria retusa and 4.75 mg/g dry wt. in *Tamarix mannifera* ,while reached to 3.73 mg/g dry wt. in *Lasiurus hirsutus* and 3.11mg/g dry wt. in *Panicum turgiodum* . This results are in agreement with that stated by Lawlor and Cornic, 2002; they reported that the accumulation of other amino acids like glycine, serine, and glutamate are known to regulate and integrate the metabolism in stressed photosynthetic tissues.

The total concentration of amino acids ranged from(76.09 mg/g d.wt) in *Tamarix mannifera* to (56.15mg/g d.wt) in *Lasiurus hirsutus*.

Roy-Macauley *et al.*, (1992) reported that water – stressed plants show a high protease activity under stressful conditions appears to be a part of an adaptive potential, since it also led to the accumulation of free amino acids which contribute to osmotic adjustment and is related to drought tolerance (Rahmanjulu *et al.*, 1994 and Dubey 1994).

1-3. Protein fractination or electrophoresis:

Sodium dodecyl sulphate polyacrylamide gel-electrophoresis (SDS-PAGE) is widely used to fractionate the proteins according to their molecular weight (Bhattly, 1982; Fullington *et al.*,1983 and Sharcbeen *et al.*, 1991).

The data presented in table (2) illustrated the results of SDS-PAGE of four studied species. The obtained result revealed that the molecular weights ranged between (98 KDa to 6 KDa) and exhibited a maximum number of (41) bands, which were not necessarily present in all different studied species, however, there were specific (3) bands present and designated as common bands no (33, 37 and 40) of about molecular weight (21,17 and 9 KDa) for both xerophytes and halophytes.

The results also indicated that the bands no. (4,7 and 12) of the molecular weight (94,73 and 55 KDa) were present in xerophytes (*Panicum turgidum* and *Lasiurus hirsutus*). The results led to the assumption that these bands represented as specific band for each corresponding habitat. The results are in agreement with those obtained by Ericson and Alfinito (1984), they reported that some protein bands were enhanced under drought stress.

The evidence of drought – resistance of plants investigated the synthesis of high molecular weight proteins. In crease of soluble proteins (high M. wt.) increases the surface exposed to water binding, as bound water is correlated to drought resistance (Larosa *et al.*, 1989 and Moons *et al.*, 1995).

In this respect, Pareek *et al.*, (1995), found that specific stress proteins accumulate in response to not just one but a number of different biotic or a biotic stress conditions for instance, HSP 90 (a group of HSPs with molecular weights in the range of 80 to 90 KDa) accumulates in response to drought stress.

It was noticed from Table (2) that the presence of the bands no. (22, 27, 35, 38, 39 and 41) associated with halophytes plants (*Tamarix mannifera* and *Nitraria retusa*) and molecular weight ranged between (38 to 6 KDa).

Table (2) Protein patterns of the studied species of xeric and halophytic plants, from up and Mid steam protein of wadi EL-Gafra.

Mol. Wt Marker KDa	*Panicum turgidum*	*Lasiurus hirsutus*	*Tamarix mannifera*	*Nitraria retusa*
	M. wt. KDa	M. wt. KDa	M. wt. KDa	M. wt. KDa
				98
97				
			95	
	94	94		
				83
			78	
66	73	73		
				67
	63			
		59		
			56	
55	55	55		
				54
			53	
			51	
			48	
				46
			44	
				42
		40		
	39			
36			38	38
		37		
				35
	33		33	
31		32		
			29	29
			28	
	25	25		25
		24		
			23	
22		22		
	21	21	21	21
		20		20
			19	19
	18			
	17	17	17	17
14			14	14
			11	11
	9	9	9	9
			6	6
Total bands	11	14	19	18

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محتوى البروتين والاحماض الامينية لبعض نباتات المراعى المتأثرة بظروف الاجهاد المختلفة

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استهدفت الدراسة:

- دراسة تاثير ظروف البيئة المتباينة على كلا من المحتوى الكلى للبروتين والاحماض الامينية التى تدخل فى تكوين البروتين وايضا تم دراسة وتحليل التفريد الكهربى للبروتين.
- وقد تم اختيار وادى الجفرة لتباين واختلاف العشائر النباتية النامية به وتم تحديد نباتين لبيئتين مختلفتين هما البيئة الجفافية والاخرى الملحية.
- وكان اختيار كلا من نبات الثمام *Panicum turgidum* ونبات *Lasiurus hirsutus* الحدة ليمثلا البيئة الجفافية حيث تم تجميعهم من اعلى الوادى بالقرب من الطريق السريع. بينما تم اختيار كلا من نبات *Tamarix mannifera* الطرفة ونبات *Nitraria retusa* الغردق ليمثلا البيئة الملحية من منتصف الوادى (شمال- شمال غرب مدينة العاشر من رمضان).

وقد اظهرت النتائج:

١- ان هناك تباين واختلاف فى تركيز محتوى البروتين الكلى بالنسبة للنباتات الجفافية كانت مراكمة البروتين اقل من النباتات التى تعيش بالبيئة الملحية.

٢- لوحظ ان عدد الاحماض الامينية المتكون منها البروتين كانت ثابتة فى ثلاثةانواع موضوع الدراسة ولكن كان هناك اختلاف فى تركيز ومكونات الاحماض الامينية الخاصة بكل نبات لكى يستطيع التأقلم مع الظروف البيئية النامى بها.

٣- اظهر التفريد الكهربى للبروتين تباين واضح بين الانواع النباتية حيث عملت النباتات التى تعيش فى بيئة ملحية على تراكم البروتين ذو الاوزان الجزيئية الصغيرة بينما راكمت النباتات الجفافية البروتين ذو الاوزان الجزيئية الكبيرة. كما لوحظ اشتراك الاربع انواع النباتية موضوع الدراسة فى بعض الحزم متشابهة الاوزان الجزيئية.