PHYSIOLOGIACL STUDIES ON EFFECT OF FOLIAR APPLICATION OF SOME MICRONUTRIENTS AND ASCORBIC ACID ON TUBEROSE *"Polianthes tuberosa* L.," PLANTES GROWN ON A SALINE CALCAREOUS SOIL.

El-Yazal, M.A
Dep. of Agic. Botany, Fac. of Agic. Fayoum, Univ., Egypt

ABSTRACT

A filed experiment was conducted to study effect of foliar spray with some micronutrients (Fe, Zn, Mn and Cu) at three concentrations, i.e.,50, 100 and 150 mg/L for each or ascorbic acid at four concentrations, i.e.,100, 200, 300 and 400 mg/L added alone or together on vegetative growth, flowering, oil yield and some chemical constituents of tuberose (*Polianthes tuberosa* L.) plants grown on a saline calcareous soil. The obtained data indicated that, all growth parameters, i.e., leaf length, leaf area, number of leaves/plant, fresh weight of leaves, dry weight of leaves were significantly increased as a result of foliar spray with micronutrients and ascorbic acid as solely or combined treatments. In additions flowering traits, i.e., flower stalk length, number of florets /spike, spike length, fresh weight of spike, dry weight of spike and number of spike per plot exhibited pronounced increase. Moreover, bulb diameter, fresh weight of bulb, number of new bulblet/plant and essential oil percentage of florets were also increased. The maximum increases in the former parameters were resulted from using micronutrients at the rate of 150 mg/L and ascorbic acid at the rate of 300mg/L alone or combined together. The same rates of micro-nutrients and ascorbic acid also led to increase the concentrations of chlorophyll a & b, caroteniods, total carbohydrates, total & reducing sugars, total indols, total free amino acids, free proline, N, P, K, Fe, Mn, Zn, Cu and vitamin C in leaves .At the same time, the activity of oxidative enzymes such as peroxidase, catalase, polyphenol oxidase and ascorbic acid oxidase were recorded the highest values by spraying with the same rates of micro-nutrients and ascorbic acid alone or together. On the other hand, the applied treatments decreased the concentrations of sodium and free phenols in leaves.

In view of these results, it has been concluded that spraying tuberose plants with the mixture of micronutrients (Fe, Mn, Zn and Cu) and ascorbic acid at the rates of 150 and 300mg/L, respectively could be counteracted the adverse conditions, particularly soil salinity and consequently producing economic flower and oil yields.

Key words: Tuberose (*Polianthes tuberosa* L.,) micronutrients (Fe, Zn, Mn and Cu), ascorbic acid, soil salinity, growth & flower parameters and chemical constituents.

INTRODUCTION

Tuberose (*Polianthes tuberosa* L., Family amaryllidaceae) is one of the most important ornamental flowering bulbs grown in Egypt due to desirable long spikes and very fragrant blossoms. The tuberose is also considered one of the natural flower oils which used in the preparation of the most expensive perfumes. This volatile oil contains geraniol, nerol, benzalcoohol, methyl benzoate, methyl salicylate and methyl anthronilate (**Yadav *et al.*, 1989**). The

drastic influence of salinity on the growth and metabolism was attributed, principally, to the enhanced Na+ uptake which causes ion excess in plant tissues (**Abbas *et al.*, 1991**). It is well established that salinity stress damages plant cells through production of reactive oxygen species including superoxide, hydrogen peroxide, hydroxyl anions and singlet oxygen **(Scandalios, 1997)**. Several authors reported that, growth and yield of tuberose plants were decreased by increasing soil salinity **(Malini and Khader, 1989; Manoly, 1989 and El-Shahat, 1993).** Previous studies have been revealed that, soil is suffering from unavailable microelements to the plant due to many factors including volatilization, fixation, microbial degradation and leaching ,in addition to soil salinity **(Mengel and Kirkby, 1979).** The simultaneous presence of salts in the root zone can be negatively influenced nutrients availability for plants as well as affect plant growth and chemical compositions. Hence, foliar application of fertilizers seems to be the best alternative method and attracted the attention of many investigators because it is very efficient in minimizing the unfavorable conditions and it had remunerated the soil microelement deficit caused by soil salinity. Microelements are needed in very small quantities for good plant growth, their deficiency or excess cause great disorders in the physiological and metabolic processes of the plant. However in Egypt, soils suffering from some micronutrients deficiencies, especially Fe, Zn, and Mn **(Fawzi, 1991)**. Many investigators such as **Kumar *et al.* (2001); Yadav *et al.* (2002); Munikrishnappa *et al.* (2002)**; **Hardeep-Kumar *et al.* (2004)**; **Selim *et al.* (2006); Abdella Ebtsam *et al.* (2006); Hassan (2007) and Somida and El-Sayim (2007)** agreed that foliar spraying tuberose (*Polianthes tuberosea* L.) plants with micronutrients (Fe, Zn Mn and Cu) increased leaf length, number of leaves/plant, fresh weight of leaves, dry weight of leaves, leaf area ,flower stalk length, number of florets/spike, spike length, fresh weight of spike ,dry weight of spike and number of spike per plot, bulb diameter, fresh weight of bulb, number of new bulblet/plant and essential oil percentage of florets, chlorophyll a& b, caroteniods, total carbohydrates, total and reducing sugars, total indols, total free amino acids and leaves content from N, P, K, Fe, Mn, Zn & Cu and decreased the free phenols.

On the other hand, some trials have been carried out to alleviate the disturbances in plant metabolism excreted by salinity stress. It has been suggested that some antioxidants which belongs ascorbic acid may help to overcome some of these inhibitory effects. The beneficial effect of antioxidant (ascorbic acid) on growth, yield and some chemical constituents of several plants was reported by several workers such as **Abd-El-Hamid *et al.* (1994); Dobariya and Mehta (1995); Rai (1997); Tarraf *et al.* (1999); El-Khayat (2001); El-Fawakhry and El-Tayeb (2003); Taha (2005); Rady (2006); El-Yazal, (2007) El-Yazal** and **Somida (2008)** on different ornamental plants including tuberose (*Polianthes tuberosa* L.) plants.

Accordingly, The present work was planned for studying effect of some micronutrients (Fe, Zn, Mn and Cu)and ascorbic acid added as foliar spray at different rates on the vegetative growth, flower or oil yields and chemical composition of tuberose *(Polianthes tuberosea* L.) plants grown on a saline calcareous soil.

MATERIAL AND METHODS

To achieve the previous objective, a field experiment was carried out during two successive seasons of 2006 and 2007 Sennouris district, Fayoum, Governorate. Bulbs of tuberose (*Polianthes tuberosea* L.) obtained from the Research Center of Medicinal and Aromatic plants in Giza, Egypt in the first season, while in the second season bulbs were obtained from the control plants of the previous season. On April 1st of each season, bulbs were sown in hills 30 cm apart entire plot (2×2) contained 3 rows (65 cm wide with 2 m length). Each plot contained 18 hills and each hill contain one plant. The experiment was designed as factorial in randomized complete block with three replications. Results were statistically analyzed using the LSD at probability level of 5% **(Gomez and Gomez, 1983).**

Some physical and chemical characteristics of the selected soil were determined according to the standard methods described by **Black *et al.* (1965) & Jackson (1973)** and the obtained results are presented in Table (1).

Table (1): Some physical and chemical analysis of the experimental soil.

Soil properties	Values	
	2006	2007
Mechanical analysis		
Coarse sand %	2.85	2.88
Fine sand %	47.15	47.62
Silt %	20.50	21.70
Clay %	29.50	27.80
Soil texture class	Sand clay loamy	Sand clay loamy
pH Soil (1:2.5)	7.81	7.76
ECe (dS/m)	7.83	7.80
CEC (meq/100g soil)	19.4	18.7
Organic matter %	1.25	1.28
Ca CO_3 %	8.55	8.42
Total N %	0.07	0.06
Soluble cations(meq/L)		
Ca^{++}	36.75	34.59
Mg^{++}	15.48	16.46
Na^{+}	27.00	27.20
K^{+}	0.60	0.70
Soluble anions (meq/L)		
CO_3^{-}	0.00	0.00
HCO_3^{-}	3.10	2.92
Cl^{-}	37.26	36.18
$SO_4^{=}$	39.47	39.85
Available nutrients (mg/kg soil)		
Fe	5.37	5.59
Mn	0.98	0.92
Zn	0.76	0.82
Cu	0.63	0.59

Plants were treated with micronutrients (M) of Fe, Zn, Mn and Cu as foliar spray with four concentrations (0,50, 100 and 150 mg /L) or ascorbic acid (A) as foliar spray with five concentrations (0, 100, 200, 300 and 400 mg/L). Triton B as a wetting agent at 0.1% was added to the antioxidant and micronutrients solutions due to their solutions were carried out till runoff. The

amount of both treatments were divided into two equal doses, and applied as basal dressing. The first dose was added at 6 weeks after sown and the second one was added at 2 weeks after the first dose. The experimental field was fertilized with calcium superphosphate (15.5% P_2O_5) at the rate of 200 kg/fed added to the soil before sowing. Nitrogen fertilizer was applied in the form of urea (46%N) at the rate of 200 kg/fed. Potassium sulphate (48% K_2O) was applied at the rate of 60 kg / fed. The amounts of N and K fertilizers were divided into two equal dose and added to the experimental plots as the recommended doses proposed by Egyptian Ministry of Agriculture.

Data recorded:

When the 1st flower of floral stalk, in each plot, seemed to be open (about 90 days after planting), the following plant parameters were determined:

1- Vegetative growth characters, i.e., leaf length (cm) from bulb, number of leaves/plant, fresh weight of leaves/plant (g), dry weight of leaves/ plant (g), leaf area (cm^2), bulb diameter (cm), fresh weight of bulb(g), number of new bulblet /plant.
2- Flowering traits, i.e., flower stalk length (cm), number of florets /spike, spike length (cm), fresh weight of spike (g), dry weight of spike (g).
3- Yield expressed as number of spike per plot and essential oil percentage of florets.
4- Chemical composition: Fresh and dried leaves (70 days of old plants for leaves and at harvest, after 120 days from planting for florets) were used for the determination of the following constituents:

Photosynthetic pigments: (chlorophyll a, b and caroteniods) as mg/100g fresh weight was extracted from fresh leaves by acetone (80%) then, their concentrations were determined according to **(Welburn and Lichtenthaler, 1984)**, total carbohydrates as g/100g dry weight was extracted by sulphuric acid (0.1 N) then determined colorimetrically by using phenol-sulphuric acid reagent according to the method described by (**Michel *et al.*, 1956**), total and reducing sugars as mg/g dry weight were determined in ethanolic extract using phosphomolybdic acid reagent as described by **(A.O.A.C., 1995),** total indols as mg/g dry weight were determined in ethanolic extract using 4-dimethyl-amino-benzaldehyde reagent as described by (**Larson *et al.*, 1962**), total free amino acids as mg/g fresh weight were determined colorimetrically in ethanolic extract using ninhydrin reagent according to the method described by **(Jayarman, 1981)**, free proline concentration as mg/g fresh weight was extracted by sulfosalicylic acid then, determined colorimetrically using acid ninhydrin and toluene reagent as described by (**Bates *et al.*, 1973**),ascorbic acid as mg/100g fresh weight was extracted by metaphosphoric acid then, determined using 2, 6-dichlorophenol indophenol as described by **(A.O.A.C, 1995)**, free phenols as mg/g dry weight were determined in ethanolic extract using Folin-Denis reagent as described by **(A.O.A.C, 1995)**, nitrogen or phosphorus % were determined according to **(A.O.A.C, 1995)**, potassium and sodium % were determined using Flame Photometer, Parkin–Elmer model 52 with acetylene burner according to (**Page *et al.*, 1982**). The following determination were make in samples of the different treatments only in the second season of 2007. Iron, zinc, manganese and copper contents as µg/g dry weight were determined by atomic absorption spectrophotometer apparatus according to **Chapman and Pratt (1961).** The total essential oil percentage of

the florets was determined at full-opening stage according to the technique of **Guenther (1961).**

Peroxidase activity was determined by the method described by **Maehly and Chance (1954), as follows:**

Half gram of fresh leaves homogenating in polytron with 4ml phosphate buffer (pH 6.0). Extracts were centrifuged for 15 minutes at 4000rpm. Peroxidase activity was measured in the supernatants using a reaction mixture consisted of 1.5ml. of phosphate buffer (pH6.0), 1.5ml. of H_2O_2 (20 volume), 1.5ml. of 0.04M catechol solution as substrates and 0.1 ml. of extract. Enzyme activities were expressed as changes in the optical density (O.D.) at470 nm 60 to 120 seconds after the substrate was added.

Catalase activity was determined by the method described by **Beers and Sizer (1952), as follows:**

Half gram of fresh leaves homogenating in polytron with 4ml phosphate buffer (pH 7.0). Extracts were centrifuged for 15 minutes at 4000rpm. Catalase activity was measured in the supernatants using 1.9 ml. of reagent grade water, 1.0 ml. of H_2O_2 and 0.1ml. of extract and the changes in the optical density (O.D.) at 240 nm were recorded for 1-2 minutes.

Polyphenol oxidase was determined by the method described by **Taneja and Sachar (1974)** as follows:

Half gram of fresh leaves homogenating in polytron with 4ml phosphate buffer (pH 6.8). The reaction mixture contained 2 ml of 1% catechol solution as substrate, 0.2ml of enzyme extract and rest of 0.05M sodium phosphate buffer pH6.8 in a final volume of 4 ml. and the changes in the optical density (O. D.) at 430 nm were recorded for 1-2 minutes.

Ascorbic acid oxidase was determined by the method described by **Dawson and Magee (1955)** as follows:

Half gram of fresh leaves homogenating in polytron with 4ml phosphate buffer (pH 6.2). The sample cuvette contained 1.0 ml. sodium phosphate buffer of (pH 6.2), 0.2 ml. ascorbic acid (10^{-3} molar), 0.1 ml. enzyme extract and 1.7 ml. distilled water. Data expressed as changes in absorption at 265 nm.

RESULTS

a. Number of days for flowering:

Data presented in Table (2) clearly indicated that spraying tuberose plants with the different rates of micronutrients and/ or ascorbic acid hastened the beginning of flowering as compared to the control plants. The number of days required for flowering were decreased with increasing the applied rates of micronutrients or ascorbic acid. The best results were obtained when tuberose plants were sprayed with the highest rate of micronutrients (150 mg/L). Such earliness reached about 4 and 3.33 days, in the first and second seasons, respectively, as compared to the control plants. Ascorbic acid treatments also

Table (2): Effect of foliar spray with some micronutrients and ascorbic acid on vegetative growth characters of tuberose plants.

Treatments	First seasons					Second seasons				
	Number of days for flowering									
	M0	M1	M2	M3	Means	M0	M1	M2	M3	Means
A0	124.50	122.33	122.00	121.50	122.58	123.66	122.50	121.50	121.00	122.16
A1	122.50	120.66	120.33	118.33	120.45	121.50	121.00	119.33	117.50	119.83
A2	120.33	117.50	116.50	116.00	117.58	121.00	118.66	117.50	116.50	118.41
A3	118.50	116.33	114.33	114.00	115.79	117.33	115.66	115.00	114.50	115.62
A4	118.00	116.00	114.00	114.00	115.50	117.00	115.33	114.00	114.33	115.16
Means	120.76	118.56	117.43	116.76		120.09	118.63	117.46	116.76	
LSD	M: 1.79	A: 1.59		MA:2.16		M: 1.86	A: 1.42		MA:2.09	
	Leaf length (cm)									
A0	60.50	61.92	66.36	71.61	65.09	63.11	63.90	65.10	68.21	65.08
A1	62.20	65.10	67.15	72.30	66.68	64.81	66.12	69.30	73.15	68.34
A2	64.30	67.18	69.21	74.10	68.69	66.18	68.20	69.90	75.19	69.36
A3	68.60	70.13	75.10	75.40	72.36	67.80	68.90	71.36	74.10	70.54
A4	68.82	70.10	75.06	75.21	72.29	67.90	68.11	71.30	74.00	70.32
Means	64.88	66.88	70.57	73.72		65.96	67.04	69.39	72.53	
LSD	M:0.88	A: 1.24		MA:1.51		M:0.81	A: 1.30		MA:1.48	
	Number of leaves / plant									
A0	45.50	50.30	56.40	64.70	54.22	48.40	55.60	58.30	62.30	56.15
A1	55.60	58.50	62.60	66.20	60.65	58.30	59.10	63.60	64.15	61.28
A2	59.80	63.10	65.60	67.40	63.97	58.90	61.60	65.90	68.30	63.67
A3	64.20	66.60	68.30	69.90	67.25	65.10	66.80	67.40	69.70	67.25
A4	64.30	66.40	68.20	69.80	67.17	65.00	66.60	66.30	68.20	66.52
Means	57.88	60.98	64.16	67.60		59.14	63.76	64.30	66.53	
LSD	M: 1.28	A: 2.41		MA: 2.29		M: 1.33	A: 2.49		MAB: 2.66	
	Fresh weight of leaves (g)									
A0	95.86	118.36	128.10	133.15	118.86	90.18	120.11	130.16	131.16	117.90
A1	125.18	129.80	136.11	139.18	132.56	128.33	131.15	134.16	136.18	132.45
A2	128.15	132.10	135.15	142.15	134.38	129.16	131.60	135.90	141.13	134.44
A3	136.21	141.25	145.28	148.80	142.88	137.63	142.11	144.25	148.36	143.08
A4	136.12	141.15	145.18	148.73	142.79	137.40	142.00	144.02	148.31	142.93
Means	124.30	132.53	145.18	142.40		124.54	133.39	137.69	141.02	
LSD	M: 0.66	A: 1.25		MA:1.33		M: 0.61	A: 1.13		MA:1. 22	
	Dry weight of leaves (g)									
A0	28.15	35.55	38.61	39.80	35.52	27.14	36.18	39.16	40.26	35.68
A1	37.60	38.30	40.95	41.66	39.62	38.66	39.63	41.15	41.36	40.20
A2	38.80	39.10	41.10	42.80	40.45	39.26	40.15	42.33	42.96	41.17
A3	40.90	42.40	43.92	45.10	43.08	42.15	43.25	43.61	45.77	43.69
A4	40.66	42.28	43.81	45.01	42.94	42.03	43.07	43.43	45.72	43.56
Means	37.22	39.52	41.67	42.87		37.84	40.45	41.93	43.21	
LSD	M: 0.40	A: 0 .81		MA:0.90		M: 0.47	A: 0.88		MA:0.94	

M: Micronutrients A: Ascorbic acid MA: Micronutrients and Ascorbic acid.

caused a significant decrease in the number of days required for flowering. The best results were obtained when tuberose plants were sprayed with the medium rate of ascorbic acid (300 mg/L). Such earliness reached about 7.08 and 6.54 days, in the first and second seasons, respectively, as compared to the control plants.

With respect to the combination between the applied micronutrients of Fe, Zn, Mn & Cu fertilization and ascorbic acid rates, it was found to have synergistic effect in producing earlier flowers. The best result of this character was obtained when plants treated with micronutrient at a rate of 150 mg/L plus ascorbic acid at a rate of 300 mg/L. which recorded 10.50 and 9.16 days, in the first and second seasons, respectively, as compared to the control plants.

b. Vegetative growth characters:

Data presented in Tables (2 &3) indicate that, tuberose plants received micronutrients and ascorbic acid as foliar application had a better growth parameters(i.e., leaf length from bulb, number of leaves/plant ,fresh weight of leaves/ plant, dry weight of leaves/ plant and leaf area)as compared to the control. Such trend was true during the two studied seasons. All tested parameters were gradually increased by increasing micronutrients and/ or ascorbic acid rates. The highest increases in growth parameters were obtained at the highest rate of micronutrients (150 mg/L.). Such increases reached 13.62 and 9.96 %, for leaf length; 16.79 and 12.49%, for number of leaves/plant; 14.56 and 13.23%, for fresh weight of leaves/ plant; 15.18 and 14.19% for dry weight of leaves/ plant and 9.55 and 7.51% for leaf area in the first and second seasons, respectively, as compared to the control plants. Ascorbic acid as foliar application, especially at the middle rate of A_3, increased leaf length from bulb by 11.07 and 8.38%, number of leaves /plant by 24.03 and 19.76%, fresh weight of leaves/ plant by 20.20 and 21.35%, dry weight of leaves/ plant by 21.28 and 22.44% and leaf area by 10.91 and 11.92% in the first and second seasons, respectively, as compared to the control plants. With respect to the interaction effect of micronutrients and ascorbic acid, data in Table (2) show that micronutrients at the rate of 150 mg/L plus ascorbic acid at a rate of 300 mg/L (M_3A_3) gave the best result for all the studied parameters. The increases recorded 24.62 and 17.41 % for leaf length from bulb; 53.62 and 44.00 % for number of leaves /plant; 55.22 and 64.51% for fresh weight of leaves/ plant; 60.21 and 68.64% for dry weight of leaves/ plant and 24.08 & 21.40% for leaf area in the first and second seasons, respectively, as compared to the control plants. The most pronounced counteracted effect on the studied soil salinity was attributed to the use of micronutrients at the rate of 150 mg/L plus ascorbic acid at the rate of 300 mg/L.

c. Flowering traits:

The obtained data in Tables (3&4) show that tuberose plants received micronutrients and ascorbic acid as foliar application had significant increases in flower stalk length, number of florets /spike, spike length, fresh weight of spike ,dry weight of spike and number of spike per plot. The best results were obtained at the highest rate of micronutrients(150 mg/L). These increments were 2.43 & 3.29% for flower stalk length; 16.44 & 13.25% for number of florets /spike; 4.92 & 4.03% for spike length; 7.05 & 4.56% for fresh weight of spike; 7.43 & 9.58% for dry weight of spike and 21.31 & 20% for number of

Table (3): Effect of foliar spray with some micronutrients and ascorbic acid on vegetative growth characters and flowering traits of tuberose plants.

Treatments	First seasons					Second seasons				
	Leaf area (cm^2)									
	M0	M1	M2	M3	Means	M0	M1	M2	M3	Means
A0	39.90	41.10	44.16	45.60	42.69	42.10	43.33	45.15	46.90	44.37
A1	42.21	43.61	46.19	46.90	44.72	44.21	45.18	47.21	48.18	46.19
A2	44.61	45.10	46.90	48.11	46.18	46.11	46.90	48.10	48.90	47.50
A3	46.01	46.91	46.98	49.51	47.35	48.11	49.18	50.16	51.11	49.66
A4	45.98	46.81	46.89	49.49	47.20	48.20	49.08	49.98	50.91	49.54
Means	43.74	44.70	46.22	47.92		45.76	46.73	48.12	49.20	
LSD	M: 0.33	A: 0 .86		MA:0.98		M: 0.41	A: 0.78		MA:1..01	
	Flower stalk length (cm)									
A0	67.15	67.65	68.25	68.82	67.96	66.38	68.16	69.15	69.68	68.34
A1	67.93	68.35	69.15	69.40	68.70	68.39	69.01	69.80	70.01	69.30
A2	68.66	68.93	69.36	69.76	69.17	68.75	69.21	69.86	70.25	69.51
A3	68.73	69.01	69.75	70.88	69.59	68.90	70.01	70.81	71.33	70..26
A4	68.71	69.06	69.80	70.61	69.54	68.76	70.36	70.63	71.15	70..22
Means	68.23	68.60	69.26	69.89		68.23	69.35	70.05	70.48	
LSD	M:0.31	A:0.57		MA:0.62		M:0.33	A: .0.59		MA:0.66	
	Number of florets /spike									
A0	22.60	23.20	24.70	25.20	23.92	23.10	23.60	24.90	25.70	24.32
A1	22.90	23.80	24.90	26.60	24.55	24 .20	24.40	25.30	26.90	25..20
A2	23.30	24.60	26.80	27.10	25.45	23.80	24.90	27.00	27.50	25.80
A3	24.15	25.70	28.30	28.80	26.73	25.60	25.90	28.70	29.10	27.32
A4	24.10	25.75	28.10	28.60	26.63	25.50	25.80	28.80	29.20	27.32
Means	23.41	24.61	26.56	27.26		24.44	24.92	26.94	27.68	
LSD	M: 0.20	A: 0 .37		MA:0.40		M: 0.22	A: 0.39		MA 0.44	
	Spike length (cm)									
A0	23.10	23.56	23.78	24.68	23.78	24.06	24.21	24.65	24.75	24.41
A1	24.16	24.65	24.87	25.21	24.72	24.38	24.69	24.96	25.81	24.96
A2	25.18	25.33	25.56	26.11	25.54	25.46	25.63	25.88	26.35	25.85
A3	25..21	25.86	26.23	26.41	25.92	25.66	25.92	26.44	26.58	26.15
A4	25..23	25.75	26.18	26.53	25.92	25.61	25.83	26.46	26.71	26.15
Means	24. 57	25.03	25.32	25.78		25.03	25.26	25.67	26.04	
LSD	M: 0.41	A: 0.83		MA: 0.93		M: 0.47	A: 0.86		MA:0.91	
	Fresh weight of spike (g)									
A0	48.33	49.56	49.98	51.10	49.74	50.11	51.12	51.69	52.01	51.23
A1	50.51	50.73	51.25	51.61	51.02	51.06	51.81	52.20	52.63	51.92
A2	50.84	51.10	51.93	54.36	52.05	51.48	52.20	52.91	54.80	52.84
A3	51.61	52.86	54.18	56.91	53.89	52.34	53.90	54.81	57.10	54.53
A4	51.61	52.80	54.21	56.83	53.87	52.30	53.86	54.93	56.95	54.51
Means	50.59	51.41	52.31	54.16		51.45	52.57	53.30	54.69	
LSD	M: 0.68	A: 0 .96		MA:2.66		M: 0.72	A: 0.92		MA:2.29	

M: Micronutrients A: Ascorbic acid MA: Micronutrients and Ascorbic acid

spike per plot over the control in the first and second seasons, respectively. Moreover, the applied ascorbic acid rates caused significant increases in flower stalk length, number of florets /spike, spike length, fresh weight of spike, dry weight of spike and number of spike per plot in the two experimental seasons as comparison with the control plants, respectively. The flowering traits were also gradually increased with increasing the rate of antioxidant concentration, however, the best results obtained with the medium rate 300 mg/L. The increase percentage over the control in this respect were 2.39 & 2.80% for flower stalk length; 11.74 &12.33% for number of florets/spike; 8.99 & 7.12% for spike length; 8.34 & 6.44% for fresh weight of spike; 8.46 & 5.57% for dry weight of spike and 23.25 & 21.35% for number of spike per plot over the control in the first and second seasons, respectively. Concerning the interaction between the applied micronutrients and ascorbic acid, it was noticed a significant difference between them in the studied two seasons. The most effective treatment that gave the greatest increases in the flowering traits was 150mg/L of micro-nutrients plus 300 mg/L of ascorbic acid, which recorded 5.55 & 7.45% for flower stalk length; 27.43 & 25.97% for number of florets/spike; 14.32 & 10.47% for spike length; 17.75 & 13.94 % for fresh weight of spike; 16.15 & 17.28% for dry weight of spike and 57.23 & 51.70% for number of spike per plot over the control in the first and second seasons, respectively. Applying micronutrients at the rate of 150 mg/L in combination with ascorbic acid at the rate of 300 mg/L was effectively due to alleviated the adverse effects of soil salinity on flowering traits.

d. Bulb and bulblet production:

Data in Table (4) showed that tuberose plants received micronutrients and ascorbic acid as foliar application significantly increased bulb diameter, fresh weight of bulb, number of new bulblet/plant in both seasons as compared to the control. The greatest increases in Bulb and bulblet production were observed when tuberose plants were sprayed with the highest rate of micronutrients (150 mg/L). Such increases reached 4.16 & 4.16% for bulb diameter, 7.40 & 7.69% for fresh weight of bulb and 31.89 & 30.00% for number of new bulblet/plant over the control in the first and second seasons, respectively. Also, applying the different rates of ascorbic acid significantly increased the bulb diameter, fresh weight of bulb, number of new bulblet /plant especially at the rate of 300mg/L, which gave the best results that reached 3.56 & 2.95% for bulb diameter; 7.19 & 6.56% for fresh weight of bulb and 36.54 & 41.83% for number of new bulblet /plant, over the control plants in the first and second seasons, respectively. The data also showed that the combination between micronutrients and ascorbic acid treatments have a significant effect on increasing bulb diameter, fresh weight of bulb, number of new bulblet/plant in both seasons. The most effective combined treatment wase obtained from used of micronutrients at the rate of 150 mg/L plus ascorbic acid at the rate of 300 mg/L added as foliar spray.

Table (4) Effect of foliar spray with some micronutrients and ascorbic acid on flowering traits and bulb & bulblet characters of tuberose plants.

Treatments	First seasons					Second seasons				
	Dry weight of spike (g)									
	M0	M1	M2	M3	Means	M0	M1	M2	M3	Means
A0	6.13	6.38	6.23	6.71	6.36	6.19	6.61	6.86	6.93	6.64
A1	6.27	6.41	6.65	6.80	6.53	6.36	6.68	6.95	6.99	6.74
A2	6.38	6.73	6.89	6.96	6.74	6.43	6.81	6.99	7.03	6.89
A3	6.82	6.79	6.92	7.12	6.91	6.71	6.95	7.12	7.26	7.01
A4	6.73	6.76	6.98	7.15	6.90	6.70	6.93	7.01	7.28	6.98
Means	6.4 6	6.61	6.73	6.94		6.47	6.79	6.98	7.09	
LSD	M: 0.14		A: 0.25		MA:0.50	M: 0.12		A: 2.27		MA:0.54
	Number of spike per plot									
A0	9.33	9.67	11.67	12.33	10.75	9.67	10.67	12.33	12.67	11.33
A1	10.33	11.33	12.67	13.33	11.91	10.67	11.67	13.00	13.33	12.16
A2	11.67	12.00	12.67	13.67	12.50	12.00	12.33	13.33	14.33	12.99
A3	12.33	12.67	13.33	14.67	13.25	12.67	13.33	14.33	14.67	13.75
A4	12.67	12.33	13.67	14.33	13.25	12.33	13.67	14.67	14.00	13.66
Means	11.26	11.59	12.80	13.66		11.46	12.33	13.53	13.80	
LSD	M: 0.94		A:0.97		MA:1.58	M:0.97		A: 0.92		MA:1.61
	Bulb diameter (cm)									
A0	3.30	3.35	3.40	3.46	3.37	3.25	3.39	3.42	3.47	3.38
A1	3.35	3.36	3.42	3.48	3.40	3.36	3.40	3.43	3.49	3.42
A2	3.38	3.42	3.46	3.52	3.44	3.39	3.44	3.47	3.51	3.45
A3	3.41	3.48	3.53	3.55	3.49	3.43	3.47	3.49	3.53	3.48
A4	3.40	3.46	3.50	3.51	3.46	3.41	3.46	3.46	3.50	3.45
Means	3.36	3.43	3.47	3.50		3.36	3.43	3.45	3.50	
LSD	M: 0.05		A: 0 .06		MA:0.08	M: 0.04		A: 0.05		MA:0.07
	Fresh weight of bulb (g)									
A0	39.60	39.75	42.25	42.41	41.00	39.55	39.69	42.60	42.71	41.13
A1	39.75	39.78	42.28	42.48	41.07	39.73	39.78	42.63	42.56	41.17
A2	42.16	42.23	42.34	45.21	42.98	42.18	42.56	42.81	45.18	43.18
A3	42.26	42.36	45.30	45.91	43.95	42.23	42.33	45.10	45.68	43.83
A4	42.23	42.31	45.21	45.26	43.75	42.20	42.31	45.05	45.60	43.79
Means	41.20	41.28	43.47	44.25		41.17	41.33	43.63	44.34	
LSD	M: 0.71		A: 1.15		MA:1.25	M: 0.68		A: 1.10		MA:1. 22
	Number of new bulblet/ plant (hill)									
A0	14.20	16.26	18.30	20.20	17.24	13.60	16.70	19.21	20.80	17.57
A1	16.61	18.20	19.90	22.10	19.20	17.06	19.66	20.20	22.60	19.43
A2	18.66	19.80	21.15	24.60	21.05	19.20	20.70	23.60	24.80	22.07
A3	20.60	22.21	25.16	26.21	23.54	22.10	24.15	26.40	27.05	24.92
A4	20.40	22.10	25.20	26.20	23.47	22.05	24.10	26.20	26.95	24.82
Means	18.09	19.71	21.94	23.86		18.80	21.06	23.12	24.44	
LSD	M: 0.66		A: 1 .15		MA:1.40	M: 0.73		A: 1.21		MA:1.33

M: Micronutrients A: Ascorbic acid MA: Micronutrients and Ascorbic acid

e. Chemical constituents:-

1- Leaf pigments:

Data recorded in Table (5) clearly showed during the successive two seasons that, foliar application of all micronutrient rates had a simulating effect on Leaf pigments of tuberose plants, however the best results were obtained at the highest rate (150 mg/L). The increments over the control treatment reached 17.17 and 18.30 for chlorophyll a; 17.39 and 19.14%, chlorophyll b and 29.16 and 33.33% for caroteniods in the first and second seasons as compared to the control plants, respectively. The data also showed that all the applied rates of ascorbic acid as user resulted in a significant increment for photosynthetic pigments (chlorophyll a & b as well as caroteniods) as compared to the control plants. A gradual increase in the pigments (chlorophyll a, b and caroteniods) are accompanied by a gradual increase in ascorbic acid rates up 300 mg/L, then decreased at the highest rate 400 mg/L .The highest increase in photosynthetic pigment concentrations were obtained by the applied of the medium rate of ascorbic acid (300 mg/L). The increases in the yield of the pigments (chlorophyll a, b and caroteniods) were 12.58 and 20.58% for chlorophyll a, 16.00 and 19.14% for chlorophyll b and 25.00 and 40.90% for caroteniods in the first second seasons over the control plants, respectively.

The interaction between micronutrients and ascorbic acid treatments was significant on the photosynthetic pigments (chlorophyll a & b as well as caroteniods) in both seasons. The highest concentration of pigments (chlorophyll a & b as well as caroteniods) was obtained when plants received micronutrients at a rate of 150 mg/L plus ascorbic acid at a rate of 300 mg /L, which caused pronounced increases recorded 34.37 & 44.26 for chlorophyll a 41.46 & 41.86 for chlorophyll b and 61.90 & 80.00% for caroteniods in the first and second seasons as compared to the control plants, respectively. Under the studied soil salinity conditions, spraying foliage of tuberose plants with micronutrients at the rate of 150 mg/L plus ascorbic acid at the rate of 300 mg/L was useful for avoiding the adverse effect (i.e. chlorosis) of soil salinity on plants which occurred on the control plants.

2 – Carbohydrates and sugars: –

Data recorded in Tables (5 and 6) clearly showed that micronutrients application had a positive effect on the chemical constituents of tuberose leaves. It is clear that, carbohydrates represented as total carbohydrates, total sugars and reducing sugars increased with increasing the rate of all micronutrient rates used. The best results were obtained with the highest rate of micronutrients 150 mg/L. The increment percentages were 12.00 and 11.52 % for total carbohydrates; 11.42 and 10.72% for total sugars; 24.72 and 25.25% for reducing sugars over the control in the first and second seasons, respectively. Ascorbic acid treatments at all applied rates had significantly increases in total carbohydrates, total and reducing sugars concentration. The carbohydrates tended to gradual increase with increasing the rate of ascorbic acid up to the 300 mg/L. The corresponding increases in total carbohydrates; total and reducing sugars at the rate of 300 mg /L were 8.70 and 9.31%, 19.12 and 18.09 % and 59.76 and 58.66%, over the control plants in the first and second seasons, respectively. Concerning the interaction between micronutrients and ascorbic acid application, the data also indicated that all combined treatments in the two experimental seasons gave significantly

Table (5): Effect of foliar spray with some micronutrients and ascorbic acid on some chemical constituents of tuberose plants.

Treatments	First seasons					Second seasons				
	Chlorophyll a (mg/g F.wt.)									
	M0	M1	M2	M3	Means	M0	M1	M2	M3	Means
A0	0.64	0.67	0.73	0.77	0.70	0.61	0.63	0.70	0.79	0.68
A1	0.67	0.75	0.78	0.80	0.75	0.68	0.73	0.79	0.82	0.75
A2	0.73	0.76	0.79	0.82	0.77	0.75	0.77	0.82	0.83	0.79
A3	0.74	0.77	0.81	0.86	0.79	0.76	0.79	0.85	0.88	0.82
A4	0.74	0.76	0.82	0.85	0.79	0.75	0.80	0.84	0.89	0.82
Means	0.70	0.74	0.78	0.82		0.71	0.74	0.80	0.84	
LSD	M: 0.05		A: 0.08		MA:0.13	M: 0.04		A: 0.06		MA:0.08
	Chlorophyll b (mg/g F.wt.)									
A0	0.41	0.44	0.47	0.50	0.45	0.43	0.45	0.48	0.52	0.47
A1	0.43	0.46	0.48	0.51	0.47	0.44	0.47	0.49	0.53	0.48
A2	0.47	0.49	0.52	0.54	0.50	0.46	0.51	0.53	0.55	0.51
A3	0.50	0.54	0.56	0.58	0.54	0.52	0.53	0.58	0.61	0.56
A4	0.50	0.53	0.57	0.58	0.55	0.50	0.54	0.57	0.59	0.55
Means	0.46	0.49	0.52	0.54		0.47	0.50	0.53	0.56	
LSD	M: 0.03		A:0.06		MA:0.07	M:0.03		A: 0.05		MA:0.07
	Carotenoids (mg/g F.wt.)									
A0	0.21	0.23	0.26	0.28	0.24	0.20	0.21	0.23	0.25	0.22
A1	0.23	0.25	0.28	0.30	0.26	0.22	0.24	0.27	0.31	0.26
A2	0.24	0.27	0.29	0.32	0.28	0.25	0.28	0.28	0.33	0.28
A3	0.26	0.30	0.30	0.34	0.30	0.27	0.29	0.32	0.36	0.31
A4	0.26	0.27	0.32	0.34	0.29	0.28	0.28	0.33	0.35	0.31
Means	0.24	0.26	0.29	0.31		0.24	0.26	0.28	0.32	
LSD	M: 0.02		A: 0 .03		MA:0.04	M: 0.01		A: 0.02		MA:0.03
	Total carbohydrates %									
A0	16.23	17.16	17.61	18.40	17.35	16.55	16.93	17.82	18.66	17.49
A1	16.75	17.90	18.30	18.60	17.88	16.98	17.83	18.56	18.93	18.07
A2	17.21	18.26	18.86	19.10	18.20	17.45	18.63	18.78	19.26	18.53
A3	17.63	18.75	19.25	19.81	18.86	17.91	18.95	19.63	20.01	19.12
A4	17.61	18.76	19.20	19.77	18.83	17.92	18.93	19.60	19.98	19.10
Means	17.08	18.16	18.64	19.13		17.36	18.25	18.87	19.36	
LSD	M: 0.85		A: 1..35		MA:1.95	M: 0.98		A: 1.28		MA:1. 86
	Total sugars mg/g D.wt.									
A0	68.73	70.77	73.80	75.81	72.27	70.76	72.78	75.83	78.84	74.55
A1	72.75	74.78	76.82	79.83	76.04	74.77	75.79	79.84	80.86	77.81
A2	76.76	81.81	83.84	85.86	82.06	78.59	83.80	85.85	88.80	84.26
A3	80.79	83.83	88.87	90.89	86.09	83.78	85.80	90.09	92.51	88.04
A4	80.28	83.62	88.58	90.27	85.68	83.29	85.53	90.00	92.10	87.73
Means	75.86	76.89	82.38	84.53		78.23	80.74	84.32	86.62	
LSD	M: 0.76		A: 0.88		MA:1.01	M: 0.63		A: 0.73		MA:1..21

M: Micronutrients A: Ascorbic acid MA: Micronutrients and Ascorbic acid.

increments for total carbohydrates, total and reducing sugars concentration. The most effective combined treatment for producing the greatest values of total carbohydrates, total and reducing sugars in the first and second seasons was micronutrients at the rate of 150 mg/L in combination with ascorbic acid at the rate of 300 mg /L in both the studied seasons.

3- Total free amino acids, total indols, free proline and ascorbic acid concentration

Data presented in Table (6) indicate that, tuberose plants received micronutrients and ascorbic acid as foliar application had a pronounced increases in total free amino acids, total indols, free proline and ascorbic acid as compared to the control plants. The greatest values of total free amino acids, total indols, free proline and ascorbic acid were obtained when tuberose plants received micronutrients at the rate of 150 mg/L in the two studied seasons. The increases were 12.66 and 10.89 % for total free amino acids; 7.53 and 6.65% for total indols; 12.00 and 15.38% for free proline and 2.52 and 9.85% for ascorbic acid over the control at the first and second seasons, respectively .Moreover, ascorbic acid at all rates used significantly increased total free amino acids, total indols, free proline and ascorbic acid as compared to the control plants. The best result for total free amino acids, total indols and free proline were obtained when tuberose plants received ascorbic acid at the rate of 300 mg /L which caused the increases of 8.29 and 6.25 % for total free amino acids, 3.64 and 3.41% for total indoles and 47.61 and 54.54% for free proline in the first and second seasons, respectively. Whereas, the maximum increase in ascorbic acid was obtained when tuberose plants received ascorbic acid at the rate of 400 mg/L which reached 42.72 and 51.31% over the control plants in the first and second seasons, respectively. With respect to the interaction between micronutrients and ascorbic acid treatments, the data also showed that the combined treatment (micronutrients at a rate150 mg/L + ascorbic acid at the rate of 300 mg/L was most effective for in increasing total free amino acids, total indols and free praline . The corresponding increase percentages were 5.29 and 5.33 mg/g F. wt.; 7.48 and 7.51 mg/100 gm F. wt. and 0.31 and 0.37 mg/g F. wt., in the two studied seasons, respectively. The maximum increase in ascorbic acid was obtained when tuberose plants received micronutrients at a rate 150 mg/L in combination with ascorbic acid at the rate of 400 mg/L which recorded 15.08 and 15.25 mg/100gm F. wt. respectively, in the two studied seasons.

Table (6): Effect of foliar spray with some micronutrients and ascorbic acid on some chemical constituents of tuberose plants.

Treatments	First seasons					Second seasons				
	Reducing sugars (mg/g D.wt.)									
	M0	M1	M2	M3	Means	M0	M1	M2	M3	Means
A0	12.10	12.55	12.76	13.60	12.75	12.46	12.83	13.01	14.55	13. 21
A1	12.75	13.36	14.25	15.66	14.00	12.86	14.21	15.33	15.92	14.58
A2	14.21	15.61	16.28	17.33	15.85	15.60	16.73	18.10	18.96	17.34
A3	17.55	18.90	22.10	22.95	20.37	18.09	19.63	22.50	23.63	20.96
A4	17.23	18.61	22.01	22.54	20.09	18.01	19.66	22.30	23.41	20.84
Means	14.76	15.80	17.48	18.41		15.40	16.61	18.24	19.29	
LSD	M: 0.23		A: 0.33		MA:0.46	M: 0.26		A: 0.38		MA:0.51
	Total indoles (mg/g D.wt.)									
A0	6.66	6.81	6.93	7.06	6.85	6.79	7.02	7.06	7.26	7.03
A1	6.75	6.79	6.96	7.26	6.94	6.86	7.06	7.16	7.30	7.09
A2	6.78	6.84	7.03	7.31	6.99	6.92	7.15	7.18	7.33	7.14
A3	6.88	6.95	7.12	7.48	7.10	7.01	7.21	7.35	7.51	7.27
A4	6.81	6.88	7.09	7.33	7.02	7.00	7.19	7.31	7.48	7.24
Means	6.77	6.85	7.02	7.28		6.91	7.12	7.21	7.37	
LSD	M: 0.09		A:0.13		MA:0.22	M:0.07		A: 0.11		MA:0.19
	Total free amino acids (mg/g F.wt.)									
A0	4.21	4.71	4.86	5.02	4.70	4.36	4.81	4.93	5.12	4.80
A1	4.36	4.78	4.91	5.08	4.78	4.69	4.83	4.98	5.14	4.91
A2	4.66	4.81	4.96	5.15	4.89	4.78	4.91	5.06	5.18	4.98
A3	4.88	4.97	5.23	5.29	5.09	4.86	5.03	5.18	5.33	5.10
A4	4.82	4.86	5.13	5.30	5 02	4.72	5.00	5.19	5.22	5.03
Means	4.58	4.82	5.01	5.16		4.68	4.91	5.06	5.19	
LSD	M: 0.38		A: 0 .48		MA:0.63	M: 0.31		A: 0.56		MA:0.70
	Free proline(mg/g F.wt.)									
A0	0.21	0.21	0.22	0.23	0.21	0.20	0.22	0.24	0.24	0.22
A1	0.23	0.24	0.25	0.25	0.24	0.24	0.26	0.26	0.27	0.25
A2	0.25	0.26	0.28	0.28	0.26	0.26	0.27	0.29	0.29	0.27
A3	0.30	0.31	0.32	0.34	0.31	0.32	0.34	0.35	0.37	0.34
A4	0.26	0.27	0.27	0.30	0.27	0.29	0.30	0.32	0.30	0.30
Means	0.25	0.25	0.26	0.28		0.26	0.27	0.29	0.30	
LSD	M: 0.02		A: 0.02		MA:0. 03	M: 0.01		A: 0.03		MA:0.04
	Ascorbic acid(mg/100gF.wt.)									
A0	10.05	10.59	10.65	10.76	10.51	9.25	9.79	10.01	10.55	9.90
A1	11.16	11.38	11.60	12.01	11.53	10.98	11.09	11.89	12.61	11.64
A2	12.60	12.73	12.96	13.50	12.94	11.93	12.55	12.81	13.60	12.72
A3	13.79	13.86	13.91	14.61	14.04	13.50	13.08	13.51	14.32	13.60
A4	14.93	14.95	15.06	15.08	15.08	14.71	14.85	15.11	15.25	14.98
Means	12.50	12.70	12.83	13.19		12..07	12.27	12.66	13.26	
LSD	M: 1.16		A: 0.49		MA:1.88	M: 1.11		A: 0.45		MA:1..81

M: Micronutrients A: Ascorbic acid MA: Micronutrients and Ascorbic acid.

4- Free Phenols and sodium concentration:

Data presented in Table (7) indicate that, tuberose plants received micronutrients and ascorbic acid as foliar application at all rates used had significant decreases in free phenols and sodium concentration as compared to the control plants in the two studied seasons. With respect to the interaction between applied micronutrients and ascorbic acid, the data also show that the combined treatment (micronutrients at the rate of 150 mg/L + ascorbic acid at the rate of 300 mg /L) was most effective for decreasing free phenols and sodium in the first and second seasons.

5– Mineral elements concentration:-

***Macro elements (N, P and K) concentrations:-**

Data in both two seasons presented in Table (7) indicate that, leaves of tuberose plants contained a high concentration of nitrogen, phosphorus and potassium under micronutrient application as compared to the control plants. Moreover, the concentration of these elements were significantly increased with increasing micronutrient rates. The maximum increases which obtained from the applied micronutrient treatment at the rate of 150 mg/L were 12.56 and 12.32% for nitrogen; 9.37 and 6.06% for phosphorus and 2.92 and 1.61 % for potassium over the control plants in the first and second seasons, respectively. The application of ascorbic acid at all rates resulted in significant increases in nitrogen, phosphorus and potassium concentration as compared with the control plants. The corresponding increases were 8.61 and 6.48%; 12.50 and 16.12 % and 4.21and 3.71% over the control plants in the first and second seasons, respectively. With respect to the interaction between applied micronutrients and ascorbic acid, the data also show that all combined treatments increased nitrogen, phosphorus and potassium concentrations as compared to the control plants. The combined treatment (micronutrients at the rate of 150 mg/L + 300 ascorbic acid at the rate of 300 mg/L). Was most effective for increasing nitrogen, phosphorus and potassium concentrations in both seasons.

***Micro nutrient (Zn, Mn, Fe and Cu) concentrations:-**

Data presented in Table (8) indicate that, leaves of tuberose plants contained high concentrations of zinc, manganese, iron and copper under micronutrients application as compared to the control plants. Moreover, the concentrations of these nutrients were significantly increased with increasing the applied micronutrients rates. The maximum increases which obtained from micronutrients treatment at the rate of 150mg/L were 6.49% for zinc; 5.07% for manganese; 2.09% for iron and 7.60% for copper over the control plants at the second season. The application of ascorbic acid at all applied rates resulted in significant increases in zinc, manganese, iron and copper concentrations as compared with the control plants. The maximum increases obtained from ascorbic acid treatment at the rate of 300 mg/L were 9.33% for zinc; 3.27% for manganese; 1.42% for iron and 8.86% for copper over the control plants at the second season. In regard to the interaction between micronutrients and ascorbic acid application, the data also show that all combined treatments in the second season gave significantly higher values of zinc, manganese, iron and copper over the control plants. The most pronounced combined treatment for producing the highest zinc, manganese, iron and copper percentages in second season was 150 mg/L of micronutrients plus 300 mg/L of ascorbic acid. That means the applied

micronutrients at the rate 150 mg/L and ascorbic acid at the rate of 300 mg/L proved to be the best treatment exhibited a pronounced counteracted effect on soil salinity under study.

6-Essential oil percentage (%):-

Data in Table (8) clearly show that the applied micronutrients treatment significantly increased essential oil percentage in the florets of tuberose plants as compared to the control plants. The highest increase in essential oil percentage was observed when tuberose plants were sprayed with the highest rate of micronutrients (150 mg/L). Such increase reached 23.72% in the second season over the control plants. Also, the different rates of ascorbic acid significantly increased essential oil percentage especially at the rate of 300 mg/L, which gave the best value (85.71%) over the control plants at the second season. The data also showed that the combination between micronutrients and ascorbic acid had a significant effect on increasing essential oil percentage in the florets of tuberose plants. The most effective combined treatment, which produced maximum value of essential oil percentage, was (micronutrients at rate 150 mg/L + ascorbic acid at the rate of 300 mg/L).

7-The enzyme activities:-

The obtained results in Table (8) revealed that tuberose plants sprayed with micronutrients had significantly higher leaves of oxidase enzymes, i.e., peroxidase, catalase, polyphenol oxidase and ascorbic acid oxidase than unsprayed ones (control). In the meantime, oxidase enzymes activity markedly increased with increasing micronutrients concentration, however, micronutrients at the rate of 150 mg/L induced the highest increase or activity for peroxidase, catalase, polyphenol oxidase and ascorbic acid oxidase. The relative increase percentages were 10.94% for peroxidase; 23.50% for catalase; 11.24% for polyphenol oxidase and 14.29% for ascorbic acid oxidase over the control plants at the second season, respectively. The application of ascorbic acid at all applied rates resulted in significant increase in peroxidase, catalase, polyphenol oxidase and ascorbic acid oxidase activityes than the unsprayed ones (control). The maximum increases obtained from ascorbic acid treatment at the rate of 400 mg/L were 26.00% for peroxidase; 29.14% for catalase; 45.39% for polyphenol oxidase and 13.95% for ascorbic acid oxidase over the control plants at the second season. The data also showed that the combination between micronutrients and ascorbic acid had a significant effect on increasing the activities of peroxidase, catalase, polyphenol oxidase and ascorbic acid oxidase in tuberose plants. The most effective combined treatment which produced a maximum increase was micronutrients at the rate of 150 mg/L + ascorbic acid at the rate of 400 mg/L resulted in an increase reached 49.33% for peroxidase; 57.03% for catalase; 60.82% for polyphenol oxidase and 31.12% for ascorbic acid oxidase at the second season over the control plants.

Table (7): Effect of foliar spray with some micronutrients and ascorbic acid on some macro-elements concentrations of tuberose plants.

Treatments	First seasons					Second seasons				
	Nitrogen (%)									
	M0	M1	M2	M3	Means	M0	M1	M2	M3	Means
A0	1.96	2.06	2.17	2.19	2.09	2.01	2.12	2.25	2.28	2.16
A1	2.05	2.08	2.30	2.31	2.18	2.09	2.16	2.31	2.36	2.23
A2	2.12	2.15	2.33	2.36	2.24	2.14	2.18	2.33	2.37	2.25
A3	2.14	2.18	2.38	2.41	2.27	2.19	2.23	2.36	2.44	2.30
A4	2.15	2.19	2.37	2.40	2.27	2.16	2.24	2.33	2.43	2.29
Means	2.07	2.13	2.31	2.33		2.11	2.18	2.31	2.37	
LSD	M: 0.09		A: 0.11		MA:0.19	M: 0.08		A: 0.09		MA:0.18
	Phosphorus (%)									
A0	0.31	0.31	0.33	0.33	0.32	0.31	0.31	0.32	0.32	0.31
A1	0.32	0.33	0.34	0.35	0.35	0.32	0.32	0.33	0.34	0.33
A2	0.33	0.35	0.36	0.36	0.35	0.34	0.36	0.36	0.36	0.35
A3	0.35	0.36	0.36	0.37	0.36	0.34	0.36	0.36	0.37	0.36
A4	0.33	0.34	0.35	0.36	0.35	0.34	0.36	0.36	0.36	0.36
Means	0.32	0.33	0.34	0.35		0.33	0.34	0.34	0.35	
LSD	M: 0.01		A:0.01		MA:0.02	M:0.01		A: 0.01		MA:0.02
	Potassium (%)									
A0	2.35	2.37	2.38	2.40	2.37	2.38	2.42	2.44	2.48	2.42
A1	2.38	2.39	2.43	2.44	2.41	2.43	2.45	2.47	2.51	2.46
A2	2.40	2.42	2.45	2.47	2.43	2.43	2.46	2.49	2.54	2.48
A3	2.43	2.46	2.48	2.51	2.47	2.46	2.51	2.53	2.54	2.51
A4	2.42	2.44	2.47	2.51	2.46	2.45	2.52	2.50	2.53	2.50
Means	2.39	2.41	2.44	2.46		2.43	2.47	2.48	2.52	
LSD	M: 0.05		A: 0 .05		MA:0.09	M: 0.04		A: 0.05		MA:1.01
	Sodium (%)									
A0	1.25	1.24	1.22	1.21	1.23	1.29	1.27	1.24	1.20	1.25
A1	1.19	1.16	1.15	1.14	1.16	1.15	1.14	1.12	1.10	1.12
A2	1.02	1.00	0.96	0.94	0.98	0.96	0.93	0.88	0.86	0.90
A3	0.84	0.82	0.81	0.80	0.81	0.80	0.79	0.78	0.78	0.78
A4	0.82	0.82	0.80	0.80	0.81	0.78	0.77	0.75	0.74	0.76
Mean	1.02	1.01	0.98	0.97		0.99	0.98	0.95	0.93	
L.S.D.	M:0.11		A:0.07		MA:0.16	M:0.06		A:0.14		MA:0.18
	Free phenols (mg/g D. wt.)									
A0	3.91	3.56	3.40	3.22	3.52	3.55	3.41	3.36	3.24	3.39
A1	3.71	3.41	3.26	3.18	3.39	3.51	3.35	3.21	3.18	3.31
A2	3.53	3.36	3.22	3.10	3.30	3.45	3.25	3.11	3.09	3.21
A3	3.41	3.25	3.14	3.06	3.21	3.28	3.20	3.06	3.01	3.13
A4	3.40	3.26	3.12	3.04	3.20	3.25	3.20	3.05	3.02	3.13
Mean	3.59	3.36	3.22	3.12		3.40	3.28	3.15	3.10	
LSD	M:0.08		A:0.18		MA:0.21	M:0.07		A:0.16		MA:0.19

M: Micronutrients A: Ascorbic acid MA: Micronutrients and Ascorbic acid.

Table (8): Effect of foliar spray with some micronutrients and ascorbic acid on some micronutrient concentrations, oxidases enzymes activity and essential oil percentage of tuberose plants.

Treatments	Second seasons					Second seasons				
	Zinc (µg/g,D. W.)					Manganese(µg/g,D. W.)				
	M0	M1	M2	M3	Means	M0	M1	M2	M3	Means
A0	77.15	80.01	82.10	82.60	80.46	120.11	124.16	128.26	128.71	125.31
A1	79.20	81.15	84.33	85.15	82.45	123.60	125.18	129.21	129.80	126.94
A2	83.20	86.26	87.10	89.50	86.51	124.10	125.70	129.80	130.11	127.42
A3	85.55	87.11	89.01	90.21	87.97	126.80	128.21	130.60	132.10	129.42
A4	85.48	87.01	89.10	90.27	87.96	126.70	128.15	130.60	132.15	129.40
Means	82.11	84.30	86.32	87.44		124.26	126.28	129.69	130.57	
LSD	M: 2.01	A: 2.51		MA:4.03		M: 3.12	A: 4.01		MA:7.43	
	Iron (µg/g,D. W.)					Copper (µg/g,D. W.)				
A0	211.10	212.15	214.25	217.06	213.64	21.05	22.15	24.21	24.60	23.00
A1	213.33	214.10	216.66	217.01	215.27	23.36	23.81	24.50	24.90	24.14
A2	214.15	214.85	217.16	218.01	216.04	24.01	24.38	25.11	25.25	24.68
A3	214.60	214.95	218.11	219.06	216.68	24.30	24.69	25.56	25.61	25.04
A4	214.61	214.93	218.12	219.05	216.67	24.30	24.70	25.53	25.56	25.02
Means	213.55	214.19	216.86	218.03		23.40	23.94	24.98	25.18	
LSD	M: 2.03	A:1.01		MA:3.06		M:2.01	A: 1.10		MA:4.02	
	Peroxidase activity					Catalase activity				
A0	100.00	114.66	118.66	120.00	113.33	100.00	120.74	137.77	140.47	124.74
A1	113.33	116.00	121.33	122.66	118.33	117.03	130.37	151.85	152.59	137.96
A2	114.66	117.33	121.33	125.33	119.66	130.37	149.62	152.59	153.33	146.47
A3	130.66	132.00	136.00	146.66	136.33	144.44	151.11	154.07	154.81	151.10
A4	130.66	137.33	140.00	149.33	139.33	148.88	154.07	155.55	157.03	153.88
Means	117.86	123.46	127.46	128.80		128.14	141.17	150.36	151.64	
LSD	M: 9.12	A: 8.20		MA:12.01		M: 11.66	A: 9.45		MA:16.36	
	Polyphenol oxidase activity					Ascorbic acid oxidase activity				
A0	100.00	111.05	116.12	120.27	111.86	100.00	112.70	117.12	119.70	112.38
A1	113.36	114.74	117.97	123.96	117.50	108.83	113.25	121.54	125.78	117.35
A2	147.46	150.23	154.37	156.68	152.18	115.10	116.39	125.41	127.80	121.17
A3	152.07	154.37	157.60	160.36	156.10	118.41	121.91	127.07	129.09	124.12
A4	152.99	155.76	159.44	160.82	157.25	119.70	123.75	130.75	131.12	126.33
Means	133.17	137.23	141.10	144.41		112.40	117.60	124.37	126.69	
LSD	M: 7.16	A: 8.96		MA:11.23		M: 5.36	A: 4.91		MA:6.96	
	Means of essential oil percentage (%)of florets									
A0	0.024	0.035	0.048	0.059	0.042					
A1	0.038	0.042	0.054	0.066	0.050					
A2	0.049	0.058	0.069	0.072	0.062					
A3	0.071	0.077	0.081	0.084	0.078					
A4	0.072	0.076	0.082	0.082	0.078					
Means	0.051	0.058	0.067	0.073						
LSD	M: 0.01	A:0.01		MA:0.02						

M: Micronutrients A: Ascorbic acid MA: Micronutrients and Ascorbic acid.

DISCUSSIONS

Spraying plants with ascorbic acid combined with some micronutrients (Fe, Mn, Zn and Cu) resulted in vigorous growth as well as highly productivity of flowers with good quality. Increasing the measured growth characters (number of leaves/plant etc...) was achieved due to the micronutrients (Fe, Mn, Zn and Cu) and antioxidant (ascorbic acid) to be easily absorbed by the plant leaves. The positive effect of the antioxidants on growth might be attributed to their positive action on enhancing cell divisions and protecting plant cells from free radicals that responsible for plant sencences, also to attributed to their effect on contracting, drought, salinity and diseases stresses as well as they have an auxinic action, consequently enhancing growth characters **(Raskine, 1992 and Elade, 1992).** Moreover, the applied micronutrient (Zn, Fe, Mn and Cu) lead to improve growth parameters because the studied soil is usually in deficient of them or they are not readily available for plants. Iron plays a role in syntheses of ribonucleic acid, reduction of nitrate to ammonia **(Russell, 1989)** ,chlorophyll synthesis **(Romheld and Maschnur, 1961),** nucleic acid metabolism and catalytic and structural roles of Fe^{++} and Fe^{+++} in plant **(Price *et al.*, 1972).**The most important function of manganese is related to the oxidation-reduction processes **(Mengel and Krikby, 1982).** It can be used as a cofactor of many enzymes that act as phosphorylated substrates; also, it plays a role in regulating the level of auxins in plant tissues by activating the auxin oxidation system **(Russell, 1989).** The essential role of Zn is related to the synthesis of tryptophan amino acid and consequently formation of auxin i.e. IAA which act as growth regulator especially in prolonging height of plants **(Devendra, *et al.*, 1999).** Moreover, the increase in dry weight of leaves/plant could be attributed to its stimulating effect on vegetative growth and physiological processes, i.e. increasing number of cells through cell division and meristematic activity of tissues. Increasing number of leaves/plant which obtained from (Table 2), micronutrients application may be attributed to the increment in dry weight of leaves. The stimulating effect of the used micronutrients on plant growth may be due to their role in electron transmission from water to chlorophyll and producing oxygen gas in the photosynthesis, in addition to their role in the nitrogen metabolism through activated nitrite reductase enzyme **(Baza, 1984).** Also, the positive increasing effect of the used micronutrients on bulb diameter may be due to their simulating effect on cell division and expansion. The beneficial effect of ascorbic acid and micronutrients on flower, oil yield and its components was mainly attributed to its positive action on enhancing growth parameters (Table 2) and photosynthetic pigments of plants leaves (Table 4). In this respect, **Al-Qubaie (2002)** stated that antioxidant, especially ascorbic acid, has an auxinic action, synergistic effect on the biosyntheses of carbohydrate and controlling the incidence of most fungi on plants which making them in vigorous states and reflects on seed yields. Besides, the induced effect of ascorbic acid, as one of vitamins, on oil content may be due to it is considered one of vitamins that are recognized to be coenzymes involved in specific biochemical reactions in plants such as oxidative and nonoxidative decarboxylations **(Robinson, 1973).** Furthermore, **Tarraf *et al.* (1999)** reported that there was an increase in essential oil content of lemongrass as a result of the foliar application with ascorbic acid. Moreover, the increase in flower ,oil yield and its components may be attributed to the metabolic role of Zn, Fe, Mn and Cu in plant. Also, the favorable effect of Zn,

Fe, Mn and Cu on yield and its components might be attributed to the increase in photosynthetic pigments concentration **(Price *et al.*, 1972)** as well as, enzyme activity, consequently enhancement of plant metabolism **Boardman (1975)**. Zinc has an essential role in carbohydrate metabolism, protein synthesis, tryptophan and IAA synthesis, since it activates number of enzymes for photosynthesis **(Gardner *et al.*, 1985** and **Marschner, 1986).** The promoting effect of ascorbic acid and micronutrients on leaf pigments concentration might be attributed to their enhancing effect on the nutritional status of tuberose plants. In this respect, **Elade (1992** and **Farag (1996)** stated that most antioxidants are responsible for accelerating the biosynthesis of various pigments leading to the increase in biosynthesis of sugars. Moreover, the stimulating effect of micronutrients on chlorophyll formation, total carbohydrates and total sugars concentration were reported by **Mohr and Schopfer (1995)** who stated that this increase may be due to that Fe, Mn and Zn enhances chlorophyll formation and consequently photosynthesis. In this respect, **Price *et al.* (1972)** reported that the basic function of zinc in plant was related to its effective role on the metabolism of carbohydrates. The increase in total indoles and total free amino acids may be attributed to the role of Zn in synthesis of tryptophane amino acid, and consequently information of natural auxin in plants i.e. indole 3-acetic acid (I.A.A.). In addition, the reduction in free phenols contrasted with the increase in total indoles i.e. indogenous promoters increased, and consequently indogenous inhibitors decreased in the leaves and led to increase plant growth parameters such as leaves length from bulb and leaves number/plant as shown in Table (2) **Sagi and Garay (1961)** showed that the effect of phenolic compounds on plant growth was contributed to their antagonism with I.A.A. activity. The increase in vitamin C may be due to the synthesize of ascorbic acid from hexose sugars and hence the adequate supply of these precursors would greatly depend on the photosynthetic activity **(Mapson, 1970).** The increase in macronutrients (N,P and K) were supported by the results of **Ahmed and Abd El-Hameed (2004)** who reported that the effect of antioxidants on producing healthy plants leads to enhance the plants to have a great ability for elements uptake . Moreover, **Devlin and Withman, (1985)** reported that, the increase in leaves N, P, K, Zn, Mn, Fe and Cu may be due to the effect of Zn on biosynthesis of auxin (I.A.A.) which promote rooting process and consequently the amounts of mineral elements absorbed and translocated into the different parts of the plant. Also, **El-Fouly and Fawzi, (1996)** recorded that the use of micronutrients as foliar spray led to an increase in root growth and thereby higher uptake of micronutrients. The positive effect of micronutrients on the activities of catalase, peroxidase ,polyphenol oxidase and ascorbic acid oxidase may be due to the effect of microelements such as(Fe and Cu) on these enzymes .In this respect **Ohkawa *et al.* (1989)** reported that ascorbic acid oxidase is a Cu-containing enzyme that catalyzes the oxidation of ascorbate to 2-dehydroascorbate with the concomitant reduction of molecular oxygen to water and may acts as a terminal respiratory oxidase or in combination with polyphenol oxidases . Plants use ascorbate as an antioxidant in an ascorbate peroxidase reaction that produces dehydroascorbate. Also, iron is either a constituent or a cofactor of many antioxidant enzymes, and can acts as a pro-oxidant factor because free or loosely bound Fe- catalyses free radical generation in the presence of reductants and peroxides through the Fenton reaction. In particular, Fe is involved in the Fe-catalysed Haber–Weiss reaction in which trace amounts of

Fe^{3+} are reduced by to produce Fe^{2+} which, in turn, reacts with H_2O_2 to form OH· (Fenton reaction). As the intrinsic constituent or metal cofactor, iron is actively involved in cellular detoxification reactions catalysed by catalase, phenolic-dependent peroxidases (non-specific peroxidases, ascorbate peroxidases and Fe superoxide dismutase, which scavenge hydrogen peroxide and superoxide, thus protecting the cell from oxidative injury. In this respect. **Ranieri *et al.* (2001)** reported that, iron deficient sunflower plants seems to affect the different peroxidase isoenzymes to different extents and to induce a secondary oxidative stress, as indicated by the increased levels of H_2O_2 Moreover, **Morre *et al.* (1987)** suggested that the ascorbic acid oxidase system might serve to generate energy-rich phosphates or alter properties of the plasma membrane. High activity of ascorbic acid oxidase is characterisitic of diverse, actively growing tissue **(Newcomb, 1951).** Also, **Marschner (1995)** reported that ascorbic acid oxidase activity decreases in copper deficient plants and it is a sensitive indicator of the copper nutritional status of a plant. Moreover, **Delhaize *et al.* (1985) and Marschner (1995)** stated that polyphenol oxidase activity was much lower in copper-deficient plants, but the activities of IAA oxidase and peroxidase were also lower. A decline in polyphenol oxidase activity with copper deficiency may be at least indirectly responsible for the delay in flowering and maturation often observed in the copper- deficient plants **(Reuter *et al.*, 1981).** The decrease in polyphenol oxidase activity was correlated with an accumulation of phenolics **(Judel, 1972).** It has been reported that catalase functions in ensuring the removal of H_2O_2, supplying free O_2, and detoxifying harmful metabolic products **(Burris, 1960).** The mode peroxidase action on the H_2O_2 substrate differs from catalase action in that peroxidase liberates free radicals rather than oxygen. These free radicals are highly phytotoxic and the use of antioxidant such as ascorbic acid were diminishing free radical formation. The accumulation of H_2O_2 may be caused changes in plant metabolism, however, it may oxidize sulfhydryl groups and inactivate IAA **(Omran 1977).** However, IAA inactivation can be reversed upon the introduction of catalase. The H_2O_2 formed by peroxidase may be scavenged by catalase. Peroxidases are a large family of oxidative enzymes which contain iron as heme and are responsible for both the scavenging of H_2O_2 by the oxidation of phenols, and their generation through the oxidation of NADH **(Polle *et al.*, 1994 and Otter and Polle, 1997).** Peroxidases which use ascorbate as a reductant (ascorbate peroxidase) are specifically involved in the H_2O_2 detoxification in chloroplasts **(Asada, 1992)** and the cytosol **(Mittler and Zilinskas, 1991).** By contrast, different functions have been reported to be carried out by 'unspecific' peroxidases by catalysing the oxidation of a wide range of phenolic substrates **(Siegel, 1993).** In the cell wall peroxidases are present in soluble, ionically-bound and covalently-bound forms and, in addition to a detoxicant role as scavengers of H_2O_2, which are involved in a number of physiological processes, and in turn regulated cell growth by catalysing the formation of cross-links between extensin, feruloyated polysaccharides and the polymerization of lignin precursors **(Abeles and Biles, 1991; Lagrimini *et al.*, 1993; Sato *et al.*, 1993; Polle *et al.*, 1994 and Christensen *et al.*, 1998).** The ability to defend plant cells against oxidative damager resulting from salinity stress is directly correlated with the level of antioxidants such as ascorbate, glutathione and a-tocopherol **(Wise and Naylor, 1987).**

Finally, from the present results, it could be concluded that the application of ascorbic acid as well as micronutrients of Fe, Zn, Mn and Cu greatly increased vegetative growth, flower and oil yield, consequently improved quality of its chemical constituents due to these elements participate in the different metabolic processes which increased syntheses of chlorophyll, carbohydrates, total free amino acids, free praline, IAA and absorption of essential nutrient. So that the used micronutrients with antioxidants could be used for producing plants with high sufficient cellular solutes enable them to overcome salinity of soils, and consequently producing greatest flower yield with high quality of tuberose plants under such soil conditions.

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دراسات فسيولوجية على تأثير الرش الورقى ببعض العناصر الصغرى وحامض الأسكوربيك على نباتات التبروز النامية فى أرض جيرية ملحية

محمد احمد سيف اليزل

قسم النبات الزراعي – كلية الزراعة – جامعة الفيوم – مصر

أجريت تجربة حقلية لدراسة تأثير الرش الورقى ببعض العناصر الصغرى (الحديد والزنك والمنجنيز والنحاس) بمعدلات صفر ، ٥٠ ، ١٠٠ ، ١٥٠ مليجرام/ لتر وحامض الاسكوربيك بمعدلات صفر، ١٠٠ ، ٢٠٠ ، ٣٠٠ ، ٤٠٠ مليجرام/ لتر كأضافة منفرده أو متحدين معا على حالة النمو الخضرى ومحصولي الازهار والزيت بالاضافة الى بعض المكونات الكيميائية لنباتات التبروز (الزنبق) النامية تحت ظروف الاراضى الجيرية الملحية **وتشير النتائج المتحصل عليها الي ما يلى:**

* أن جميع الصفات الخضرية مثل طول الاوراق، عدد الأوراق/ نبات، الوزن الطازج والجاف للأوراق، مساحة سطح الورقة قد زادت زيادة معنوية باستخدام كل من العناصر الصغرى وحامض الاسكوربيك كأضافة منفردة او مشتركة رشا على النباتات وقد امتد هذا التأثير الايجابى علىجميع الصفات الزهرية (طول الشمراخ الزهرى، عدد الزهيرات على الحامل النورى، طول الحامل النورى، والوزن الطازج والجاف للحامل النورى، عدد الشماريخ الزهرية ، محصول الازهار) بالاضافة الى قطر البصلة، الوزن الطازج للبصلة، عددالبصيلات الجديدة، نسبة الزيت الطيار للزهيرات.
* سجلت أعلى زيادة فى الصفات السابقة عند ا ستخدام معدل ١٥٠ مليجرام/ لتر من العناصر الصغرى و ٣٠٠ مليجرام/لتر من حامض الاسكوربيك بصورة منفردة او مشتركة رشا على النباتات..
* تشير النتائج ايضا الى ان نفس المعدلات السابقة من العناصر الصغرى وحامض الاسكوربيك كل بمفرده أو باتحادهم معا قد ادت الى زيادة تركيز كلوروفيل أ و ب والكاروتينيدات، والكربوهيدرات الكلية والسكريات الكلية والمختزلة والأندولات الكلية والأحماض الأمنية الحرة الكلية والبرولين والنتروجين والفسفور والبوتاسيوم والحديد والمنجنيز والزنك والنحاس وفيتامين ج فى الاوراق. كذلك زيادة نشاط انزيمات الاكسدة مثل انزيم البيروكسيديز والكتاااليز والبولى فينول اوكسيديز والاسكوبيك اسد اوكسيديز. وعلى العكس من ذلك فقد أدت كل المعاملات الى نقص الفينولات الحرة وعنصر الصوديم فى الاوراق.
* **فى ضوء تلك النتائج** فأن هذة الدراسة تلقى الضوء على اهمية دور العناصر الصغرى ومضادات الاكسدة مثل حامض الاسكوربيك (كمواد طبيعية وامنة) كبديل لمنظمات النمو المختلفة الضارة على زيادة تحمل نباتات التبروز للملوحة. كما تشير النتائج الى ان استخدام مخلوط العناصر الصغرى بمعدل١٥٠ مليجرام/ لتر و حامض الاسكوربيك بمعدل ٣٠٠ مليجرام/ لتر بمفردهم أو بأ تحادهم معا رشا على النباتات كاف لانتاج نباتات تتحمل الظروف المعاكسة من ملوحة التربة وانتاج محصول اقتصادى من الازهار والزيت فى ظل هذة الظروف.