

EFFECT OF YEAST EXTRACT, AMINO ACIDS AND CITRIC ACID ON PHYSIOANATOMICAL ASPECTS AND PRODUCTIVITY OF TOMATO PLANTS GROWN IN LATE SUMMER SEASON

**S. A. El-Desouky⁽¹⁾, Faten H. M. Ismaeil⁽¹⁾, A. L. Wanas⁽²⁾,
E. L. E. Fathy⁽³⁾ and M. M. Abd El -All⁽¹⁾**

⁽¹⁾ Agric. Botany. Dep. Fac. of Agric. Benha Univ.

⁽²⁾ Agric. Botany. Dep. Fac. of Agric. Mansoura Univ., Domiat Branch.

⁽³⁾ Vegetable Dept., Hort. Inst., Agric. Res. Center.

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ABSTRACT: *Two field experiments were conducted at the Experimental Farm Station of Hort. Faculty of Agriculture, Benha University, during late summer seasons of 2006 and 2007 on tomato (*Lycopersicon esculentum*, Mill.) Cv. Super strain B.*

Treatments were as follow, Yeast extract at 15 and 30 ml/L, Amino acids at 1.5 and 3ml/L., Citric acid at 2.5 and 5 g/L. and Control (water treated). The obtained results clearly showed that yeast extract at 30 ml/L gave the highest values for each of number of flowers, total fruits per plant and percentages of fruit setting. Most applied treatments were effective and highly increased the fertility of pollen grains, total yields, total fruits per plant, weight of total fruits per plant, fresh and dry fruit weight, length, diameter and shape index per plant in treated plants compared with control.

The quality characteristics of tomato fruits i.e., vitamin C, titratable acidity and total soluble solids as well as minerals content were increased with different treatments. The most effective treatments of tomato plant were yeast extract at 30 ml / L and amino acids at 3 ml / L

Different applied treatments were highly increased photosynthetic pigments content, minerals concentrations (N, P, K, Mg mg/g d.w and Fe ppm) also crude protein, total sugars, total carbohydrates, total phenols and total amino acids in tomato leaves, minerals concentrations (N, P, K, Ca mg/g d.w and Fe ppm) in fruit as well as levels of endogenous auxins, gibberellins and cytokinins (in shoot) meanwhile, abscisic acid was decreased as compared with the untreated plants. at 70 days after transplanting during the assigned season.

The application of various treatments induced reduction in enzymatic antioxidants activity (i.e., peroxidase, catalase and superoxide dismutase) as compared with control plant those of activity could be attributed to antioxidant direct effects of used treatments on scavenging toxic radicals.

Vigorous growth of tomato plant treated with different applied treatments was positively correlated with different anatomical responses of stems and leaves. Since, different applied treatments increased stem anatomical features e.g. stem thickness, increment of stem thickness was accompanied with increases in most of its anatomical features, i.e., thickness of epidermis, thickness of cortex and thickness of parenchymatous pith.

Moreover, increased thickness of midvein, lamina, upper epidermis, lower epidermis, thickness of spongy tissue and palisade tissue as well in tomato leaf. Furthermore, increasing dimentions (length and width) of vascular bundles, thickness of both phloem and xylem tissues and number of xylem vessels.

Finally, it could be concluded with spraying tomato plant (under late summer/ heat stress conditions), 5 times with yeast extract at 30 ml/L for maximizing fruit yield.

Keywords: *Tomato plant; High temperature stress; anatomical study; yeast extract; amino acids and citric acid.*

INTRODUCTION

The tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and economical vegetables. It is grown in nearly all home gardens and by a large percentage of market gardeners. It is known as a favorite vegetable crop, rich in antioxidants, vitamins and minerals for human diet. In Egypt, the late summer market tomato crop is yielded from transplants raised from open field during May up to July. During this period, temperature can exceed 35°C under field condition resulting in either non-uniform growth and poor fruit yield or even completely failure of tomato cropping in a great part of the cultivated area (Pressman *et al.*, 2002). Sato *et al.*, (2006) reported that under high temperatures, fruit set in tomato plants failed due to disruption of sugar metabolism and transport during the narrow window of male reproductive development.

Tomato (*Lycopersicon esculentum* Mill.) production is limited by high day-time temperatures. Mean temperature is more critical than night-time temperature per set. At daily mean temperatures of 29°C, fruit number, fruit weight per plant and seed number per fruit were markedly decreased compared with those at 25°C continuous exposure of tomato to high temperatures (day/night temperatures of 32/26°C) markedly reduced number of pollen grains per flower and decreased viability (Pressman *et al.*, 2002).

Heat stress due to increased temperature is an agricultural problem in many areas in the world. Transitory or Constantly high Temperatures cause an array of morphological, anatomical, physiological and bio chemical changes in plants , which affected plant growth and development (Wahid *et*

al., 2007). Higher temperature stress accelerate the formation of toxic ROS (reactive oxygen species) within plant tissues or impair the normal defense mechanisms that protect tissues from ROS toxic effect. Such stress induce higher O₂ photo reduction within chloroplasts or electron transport disturbance, and donation of electron to O₂ within mitochondria , all led to generation of toxic ROS.(Elestner and Osswald, 1994).

Few years ago, this was the case, where extreme higher temperature were prevailed in Egypt during the summer months. Consequently, tomato plant chlorosis, abscission of chlorotic mature leaves, poor fruiting and bad fruit quality were frequently observed (heat stressed plants) during this period. Recently, new rooted physiological understanding and information were reliable, accordingly new effective techniques might be investigated to induce plant tolerability.

Also, it was demonstrated that an internally inducible, oxidative stress, the internal generation of reactive oxygen species (ROS, toxic oxygen free radicals), this know as the main factor beyond heat and other stresses related disturbances. Also, it was stated that oxidative stress tolerance considered as an important factor for all stresses tolerability (Howarth, 2005).

Antioxidants, i.e., citric acid due to their molecules auto (ox-redox) properties act as cofactors for some specific enzymes i.e., dismutases, catalases, peroxidases and those catalyzed breakdown of the toxic H₂O₂, OH and O₂ radicals (Fathy *et al.*, 2003).

Photosynthesis, one of the most heat sensitive processes, can be completely inhibited by high temperature before other symptoms of the stress are detected (Camejo *et al.*, 2005). This photosynthesis decrease could result from structural and functional disruptions of chloroplasts and reduction of chlorophyll accumulation under high temperature stress (Dekov *et al.*, 2000).

The aim of this study was to study the possibility of using some natural materials (yeast extract, amino acids and citric acid) effects on some physiological and anatomical characteristics as well as flowering, yield and fruit quality of treated tomato plants compared with control by enhancing the internal metabolic defensive processes of tomato plant against the higher temperature adverse effects towards maximizing its growth and productivity.

MATERIALS AND METHODS

Two field experiments were conducted at the experimental farm station of the Faculty of Agric., Benha Univ., during late summer seasons of 2006 and 2007 which, during this period, temperature can exceed 35°C under field conditions.

At the beginning of May 1st during the two seasons, after 45 days from seeds sowing. Seedlings were transplanted on 1st of May in open field at 30cm apart on one side of ridge 3.5 m long and 1m wide, experimental unit area was 10.5 m². Randomized complete block design in three replicates was adopted.

Plants were sprayed 5 times with different assigned treatments, the first one was 20 days after transplanting and repeated each 15 days. All cultural practices were performed as recommended. Treatments were as follows:

1. Yeast extract at 15 and 30 ml/L.
2. Amino acids mixture at 1.5 and 3 ml/L.
3. Citric acid at 2.5 and 5g /L. and 4. Control (water treated).

1-Preparation of Yeast extract:

The dry pure yeast powder was activated by using sources of carbon and nitrogen with the ratio of 6:1 (Barnett *et al*, 1990). This ratio is suitable to get the highest vegetative production of yeast (each ml yeast contained about 12000 of yeast cells). Then the media was frozen and thawed directly before usage. Tween- 20 was added as a spreading agent for all treatments.

The yeast extract used in the present study was analyzed for phytohormones, mineral elements "macro and micro", amino acid (Arginine,1.99; Histidine, 1.63; Isoleucine, 1.31; Leucine, 2.09; Lysine, 1.95; Methionine, 0.72; Pheylalanine, 1.01; Threonine, 1.09; Tryptophan, 0.45; Valine, 1.19; Glutamic acid, 1.00; Serine, 1.59; Aspartic acid, 1.33; Cystine, 0.23; Proline, 1.53 and Tyrosine, 1.49), total carbohydrates, reducing sugars as glucose, enzymes and Vitamins(Vitamin B1, 1.23; Vitamin B2, 1.31; Riboflavin, 2.96; Nicotinic acid, 25.89; Panthothenic acid, 13.56; Biotin, 0.09; P-amino benzoic acid, 6.23; Vitamin , B6,1.25; Folic acid, 2.36; Thiamin, 2.71; Pyridoxine, 2.90 mg/ 100g fresh weight and Vitamin B12, 1.53 and Inositol, 202.1 (µg/100g) by Mahmoud (2001)

2- Amino acids composition:

Spanish compound known as delfan 10% L-α free amino acids was used as a stimulative compound source for amino acids mixture . Delfan was a commercial containing amino acids mixture (mg/ 100ml)

Aspartic, 2.3; Glycine, 4.6; Threonine, 1.2; Tyrosine, 0.9; Glutamic, 4.2; Histidine, 0.3; Alanine, 2.5; Cystine, 0.2; Valine, 1.8; Methionine, 0.2; Iso-Leucine, 1.1; Leucine, 2.1; Phenyl-alanine, 1.1; Hidroxiproline, 2.7 Serine, 2.8; Arginine, 2.6; Proline, 2.8 and Lysine, 1.1; amino nitrogen, 1.4% as well as organic matter, 18.4%.

The compound was obtained from Techno Green Comp. Group Cairo, Egypt.

3-Climatologically data:

The monthly air temperature mean during the two growing seasons (2006 and 2007) in the cultivated area are indicated in Table (1).

Maximum and minimum air temperature were recorded monthly after Shebeen El-Kanater weather station and indicated in Table (1).

Table (1): Monthly air temperature mean in Shebeen El-Kanater during summer seasons of 2006 and 2007.

Months	Air temperature °C			
	2006		2007	
	Maximum	Minimum	Maximum	Minimum
May	31.5	18.7	31.1	18.9
June	35.0	20.8	36.9	21.7
July	36.6	22.5	38.4	23.5
August	37.9	23.7	37.3	23.1
September	35.8	22.0	35.0	24.4

I-Reproductive aspects

-Flowering characteristics:

For studying the flowering behavior of the various treatments in tomato, nine plants per each treatment were randomly taken, labeled and the following data were recorded:

a) Total number of flowers / plant.

b) Abscission percentage: Was calculated according to the equation:

$$\text{Abscission \%} = \frac{\text{Total No. of formed flowers/plant} - \text{Total No. of setted fruits/plant}}{\text{Total No. of formed flowers / plant}} \times 100$$

c) Pollen grains fertility:

Pollen fertility was estimated by the inspection and counting of fertile and non-fertile pollen grains mounted in dilute iodine solution and the second way was by the germination of pollen grains on a cultural media (Shahine 1961).

d) Fruit setting percentage:

Was calculated according to the following equation:

$$\text{Fruit setting \%} = \frac{\text{No. of setted fruits / plant}}{\text{No. of formed flowers / plant}} \times 100$$

-Fruit yield and yield components:

All harvested fruits (in the marketable color i.e., at the pink to light red stages) of tomato fruits from each treatment all over the season were used to calculate the following parameters:

- a) Total number of fruits / plant.
- b) Total yield (kg) / plant
- c) Quality characteristics of tomato marketable fruits:
 - 1- Average fruit weight (gm).
 - 2- Average fruit dry weight (gm).
 - 3- Fruit diameter (cm).
 - 4- Fruit length (cm).
 - 5- Fruit shape index (L/D): It was calculated as the ratio between the length and the diameter (L/D) of the fruit.
 - 6- Total soluble solids (T.S.S.): Total soluble solids (T.S.S.) was measured in the juice of tomato fruits by using a hand Refractometer.
 - 7- Vitamin C and titratable acidity: Both Vitamin C concentration and titratable acidity were determined in fresh tomato fruits according to the method described by the A.O.A.C. (1990).

II-Chemical analysis in leaves and fruits:

The samples were randomly chosen from tomato leaves at 70 days after transplanting and in fruits at the marketable stage at the midseason during 2007 season to determine some chemical analysis i.e., Photosynthetic pigments, total nitrogen and crude protein, Phosphorus, Potassium, Calcium, Magnesium, Iron, Total carbohydrates and sugars, Total free amino acids, Endogenous Phytohormones, Total phenols and Assay of enzymes activities which determined and calculated according to the methods described by A.O.A.C. (1990).

III- Anatomical studies:

Specimens of stems were taken from the 5th apical internode of the main stem while, those of the leaves were taken from the certain leaflet of the 4th apical leaf on the main stem.

These vegetative specimens were then killed and fixed in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%), washed in 50% ethyl alcohol, dehydrated in a series of ethyl alcohols 70, 90, 95 and 100%, infiltrated in xylene embedded in paraffin wax with a melting point 60-63°C, sectioned 15 microns in thickness for stem and 20 microns for the leaf (Sass, 1951), stained with the double stain method (Fast green and safranin), cleared in xylene and mounted in Canada balsam (Johanson, 1940). Four sections treatment were microscopically inspected to detect histological manifestations of noticeable responses resulted from treatments. Counts and measurements (μ) were taken using a micrometer eye piece. Averages of readings from 4 slides / treatment were calculated.

IV-Statistical analysis:

Data of flowering and yield characteristics were statistically analyzed and the means were compared using the Least Significant Difference test (L.S.D) at 5% and 1% levels according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

1. Reproductive characteristics:

1.1. Flowering characteristic:

As shown in Table (2) the number of flowers/plant, it was significantly increased with most applied treatments during the two grown seasons. The exception was that significant increase existed with each of yeast extract at 30 ml/L. during 2006 season, yeast extract at 30 ml/L. and amino acids at 1.5 ml/L during 2007 season.

On the other hand, abortion of flowers was decreased. Since, percentage of flower abscission was decreased to reach the 5% level of significance with different applied treatments except that significant decrease with yeast extract at 30 ml/L. Again, it could be concluded that reduction in flowers abscission percentages of in turn enhancement of fruit setting obtained with treatment may be due to increase of total carbohydrates, protein and mineral concentrations in the leaves (source) as well as the endogenous auxins, especially at full blooming and setting stages.

1. 2. Pollen grains fertility:

Data in Table (2) clearly indicate that, different applied treatments highly increased the fertility of pollen grains in treated plants compared with control. Also it could be noticed that the yeast extract at 30 ml/L gave the highest fertility. The above mentioned results could be directly reversed upon the high percentages of fruit setting as previously mentioned (Table 2). Since yeast extract treatment, also gave the lowest percentage of sterile pollen grains. Moreover, the above mentioned results are of great interest, since fruits setting, number, as well as total fruit yields are completely depending on them.

In tomato, reproductive processes were adversely affected by high temperature, which included meiosis in both male and female organs, pollen germination and pollen tube growth, ovule viability, stigmatic and style positions, number of pollen grains retained by the stigma, fertilization and post-fertilization processes and growth of the fertilized embryo. In this respect, Pressman *et al.*, (2002) reported that continuous exposure of tomato to high temperatures (day/night temperatures of 32/26°C) markedly reduced the number of pollen grains per flower and decreased viability.

Table (2): Effect of yeast extract, amino acids and citric acid on flowering, fruit setting, flower abscission and pollen grains fertility of tomato (*Lycopersicon esculentum*, Mill.) plants during 2006 and 2007 late summer seasons.

Characters Treatments		No. of flowers / plant		Total fruits (No./plant)		Fruit setting (%)		Abscission (%)		pollen grains fertility(%)	
		Seasons		Seasons		Seasons		Seasons		Fertility	Sterility
		2006	2007	2006	2007	2006	2007	2006	2007	2007	
1. Yeast extract at 15 ml/L.		67.67	74.67	23.33	19.00	34.53	25.54	65.47	74.46	38.71	61.29
2. Yeast extract at 30 ml/L.		87.67	79.33	27.67	26.67	31.58	33.74	68.43	66.26	44.88	55.12
3. Amino acids at 1.5 ml/L.		80.00	64.33	23.33	20.00	29.18	31.59	70.82	68.41	34.62	65.38
4. Amino acids at 3 ml/L.		66.33	74.33	21.33	23.00	32.22	30.97	67.78	69.03	36.45	63.55
5. Citric acid at 2.5g/L.		73.33	79.00	21.67	22.33	29.59	28.39	70.41	71.61	33.24	66.76
6. Citric acid at 5 g/L.		74.33	79.67	21.33	22.67	28.74	28.58	71.26	71.42	30.84	69.16
7. Control		56.33	59.67	12.00	11.00	21.61	18.99	78.39	81.01	19.23	80.77
L.S.D.	0.05	6.30	10.46	1.77	2.49	3.82	5.57	3.82	5.57	4.07	4.12
	0.01	8.48	14.08	2.38	3.35	5.14	7.50	5.14	7.50	6.44	5.51

1.3. Fruit yield and yield components:

Data presented in Tables (3) clearly show that fruit yield and its components of tomato were highly increased as affected by different applied treatments in relatively similar fashion as previously mentioned. All applied treatments significantly increased fruit setting, No. of fruits / plant and fresh and dry weight g) / fruit and in turn, greatly improved the total fruit yield (kg / plant) compared with the control during the two growing seasons. The highest total fruits No./ plant and total yield Kg/ plant were obtained with yeast extract at 30 ml/L. treatment.

Also, fruit length (cm) and diameter (cm) were greatly differed among most of treatments and control especially at 5% level of significance. Herein, it was observed that the stimulative effect of such treatment on tomato fruit yield was mainly due to their promotional effect on fruit setting and number of fruits/plant rather than fruit weight.

This also could be due to the pronounced enhance able effect of the same treatments on growth behavior, N, P and K content, metabolic activity (chlorophyll and carbohydrate content), and the anti-oxidant bioconstituents, i.e. carotenoids and phenols content. All of them positively correlated with fruit yield. Once again, plants of these treatments were of the highest carbohydrates content might be exported sufficient sugars at early stages, those which essentially required for fruit setting activities, especially under stress condition (Fathy *et al.*, 2003).

1.4. Some bioconstituents of tomato fruits:

Data in Table (3) indicate that some constituents (total acidity, Vitamin C and total soluble solids)and minerals concentrations were greatly increased with most applied treatments during the late summer of 2007 season compared with the control. Increasing such constituents in tomato fruits consider very important since, tomato is one of the highly important foods in human nutrition for its highly nutritive value. It is rich in vitamins A and C, in addition to its value to human healthy, contributed to tomato acidity. Hence, the applied treatments improved the quality of tomato fruits by increasing their concentrations of total soluble solids, Vitamin C and the titratable acidity.

2. Chemical composition:

2.1. Photosynthetic pigments:

Data presented in Table (4) clearly indicate the effect of different applied treatments in increasing each of chlorophyll a, b and carotenoids concentrations compared with the control at 70 days after transplanting during both seasons. Yeast extract at 30 mg/L was more efficient in this respect followed by citric acid at 5 g/L. These results are of great interest,

Table (3): Effect of yeast extract, amino acids and citric acid on total fruits No., total yield / plant , fresh and dry weight/fruit, Fruit diameters, Fruit shape index, some minerals, vitamin C, total soluble solids (%) and total acidity (%) of tomato (*Lycopersicon esculentum*, Mill.) plants during 2006 and 2007 late summer seasons.

Characters Treatments	Total yield (kg/plant)		Fruit fresh weight (g)/fruit		Fruit dry weight (g)/fruit		Fruit diameters				Fruit shape index (L/D)		Minerals mg/g dry weight				Fe ppm	Vitamin C (mg/100 cm ³ juice)	Total soluble solids (%)	Total acidity (%)	
							Length (cm)		Diameters (cm)				N	P	K	Ca					
	Seasons		Seasons		Seasons		Seasons		Seasons		Seasons		Season 2007								
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007									
1. Yeast extract at 15 ml/L.	1.55	1.45	69.55	76.35	4.09	4.06	5.75	6.24	5.22	5.16	1.10	1.21	39.40	6.60	29.87	3.77	227.50	20.03	4.47	0.48	
2. Yeast extract at 30 ml/L.	1.82	1.82	66.00	68.23	4.60	4.76	6.28	6.23	5.52	5.27	1.14	1.18	29.50	6.03	28.84	4.25	304.00	21.95	4.55	0.40	
3. Amino acids at 1.5 ml/L.	1.59	1.43	68.04	72.23	3.56	3.54	6.27	6.54	5.31	5.64	1.18	1.16	29.50	6.60	32.96	2.95	271.20	19.72	4.03	0.45	
4. Amino acids at 3 ml/L.	1.46	1.64	68.49	71.70	3.90	3.84	6.12	6.52	5.32	5.28	1.15	1.23	32.00	8.65	28.84	3.02	264.00	20.67	3.45	0.46	
5. Citric acid at 2.5g/L.	1.63	1.58	75.06	71.20	4.18	4.18	6.50	6.50	5.45	5.34	1.19	1.22	29.50	6.19	26.78	3.82	365.50	20.76	3.74	0.49	
6. Citric acid at 5 g/L.	1.61	1.36	75.37	75.69	4.35	3.91	6.50	7.10	5.30	5.15	1.23	1.38	27.10	5.62	29.87	4.35	363.00	21.54	3.36	0.49	
7. Control	0.66	0.64	55.26	60.39	3.00	2.84	5.60	5.94	5.00	5.00	1.12	1.19	24.60	5.13	22.66	2.51	212.50	17.43	3.35	0.38	
L.S.D.	0.05	0.14	0.21	5.62	6.87	0.40	0.51	0.64	0.59	0.38	0.38	0.13	0.14								
	0.01	0.19	0.29	7.57	9.25	0.54	0.68	0.86	0.80	0.51	0.51	0.17	0.19								

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Table (4): Effect of yeast extract, amino acids and citric acid on photosynthetic pigments concentration(in 2006- 2007 seasons), some minerals (mg/g D.W.), total carbohydrates, sugars, crude protein, amino acids and phenols concentrations in tomato (*Lycopersicon esculentum*, Mill.) leaves at 70 days after transplanting during 2007 late summer season.

Characters Treatments	Photosynthetic pigments mg/g fresh weight								Minerals mg/g dry weight				Fe ppm	mg/g dry weight				
	Chl. a		Chl. b		Chl. a+ b		Carots.		N	P	K	Mg		Total carbohydrates	Total sugars	Crude protein	Total amino acids	Total phenols
	2006	2007	2006	2007	2006	2007	2006	2007										
1. Yeast extract at 15 ml/L.	1.14	1.19	0.67	0.73	1.81	1.92	0.63	0.75	46.80	3.61	18.54	5.80	235.10	482.30	42.30	292.5	15.79	3.88
2. Yeast extract at 30 ml/L.	1.45	1.52	0.85	0.91	2.30	2.43	0.82	0.86	51.70	3.36	29.57	6.26	265.20	553.00	44.12	323.13	16.43	4.49
3. Amino acids at 1.5 ml/L.	1.03	1.12	0.54	0.70	1.57	1.82	0.60	0.64	32.00	3.57	16.48	4.26	285.50	452.00	32.20	200.00	12.34	2.90
4. Amino acids at 3 ml/L.	1.17	1.12	0.60	0.70	1.77	1.82	0.62	0.64	44.30	2.79	16.48	4.86	282.00	463.50	34.37	276.88	10.82	2.82
5. Citric acid at 2.5g/L.	1.15	1.19	0.60	0.61	1.75	1.79	0.65	0.66	46.60	4.14	18.54	6.33	283.50	507.10	38.95	291.25	11.51	2.76
6. Citric acid at 5 g/L.	0.93	1.05	0.46	0.52	1.39	1.57	0.57	0.66	41.80	3.32	27.51	6.30	287.30	498.80	37.16	261.26	10.29	3.12
7. Control	0.89	0.93	0.67	0.45	1.56	1.38	0.49	0.58	27.10	2.42	16.48	3.60	158.30	335.50	23.11	169.38	8.86	1.09

because they are lightly considered direct reason for the more dry matter production and distribution in shoots of tomato plants as affected by different applied treatments. It was obvious that control plants were greatly stressed. This might be due to either poor synthetic capacity or due to degradation of chlorophyll as result of heat /oxidative stress effects Camejo *et al.*, (2005) they reported that under high temperatures, degradation of chlorophyll a and b was more pronounced in developing leaves.

2.2. Minerals concentrations at 70 days after transplanting :

Data in Table (4) illustrated that all applied in leaves treatments were effectively increased N, P, K and Mg concentrations of treated plants compared with those of the untreated plants .Again, most effective treatments was yeast extract at 30 ml/L followed by citric acid at 5 g/L. The simulative effect of these treatments might be due to the higher mineral metabolic requirements to face the higher obtained vigorous growth and yield potentialities there by more minerals uptake and translocation .

The most effective treatment which led to maintained the highest concentrations of Fe was citric acid at 2.5 and 5 g/L followed by amino acids at 1.5 and 3 ml/L, respectively.

Additionally, the main function of anti-oxidants is their protective effect of cell membranes and their binding transporter proteins (H⁺-ATP-ase membrane pump), maintained their structure and function against the toxic and destructive effects of ROS during stress, in turn, more absorption and translocation of minerals. Here, it could be concluded that increase of leaf area (EI- Desouky *et al.*, 2009) and photosynthetic pigments as well as increment of dry matter accumulation in leaves reverse the stimulatory effects of these elements on the efficiency of photosynthesis process, hence more photosynthates being created as well as enhancement of minerals translocation from roots to leaves.

2.3. Total carbohydrates, Sugars and crude protein concentrations in leaf at 70 days after transplanting:

Data in Table (4) clearly indicate that the most effective treatments which maintained the highest carbohydrates was amino acids at 1.5 ml/L followed by yeast extract at 30 ml/L. In this respect, increasing of total carbohydrate with different applied treatments consider as a direct result of increasing both photosynthesis rate and efficiency. Also, that was preceded with large photosynthetic area and high concentration of photosynthetic pigments Table (4) as well under the treatment of various treatments but reached its maximum with yeast extract at 30 ml/L. Such promotional effect of applied anti-oxidant on carbohydrates concentrations could be due to their similar effect on photosynthetic pigments, and number of leaves, surfaces of photo-

assimilation. Thereby the capacity of CO_2 fixation and carbohydrates synthesis (Añon *et al.*, 2004)), also citric acid might be involved in ATP synthesis and decreased the destruction of carbohydrates (Fathy *et al.*, 2003). The simulative effect of the yeast extract, and citric acid might be due to their anti-oxidantal scavenging effect to protect chloroplasts and prevent chlorophyll degradation by the toxic reactive oxygen radicals which internally generated during high temperature stress. Lu and Huang (2003) suggested that plant stress tolerance will depend on the intrinsic anti-oxidation system in leaf cells.

As for the total sugars and their fraction, data in Table (4) exhibited their dominant increases with all applied treatments at the two assigned times of determination. Also, yeast extract at 30 and 15 ml/L treatments gave the highest values of their concentrations. Yet, it was followed by amino acids at 3 ml/L.

As for protein concentration in Table(4), it could be noticed that it behaved the same as in case of elements since the different applied treatments increased this concentration compared with the control These results are in agreement with those reported by Marschner (1995),

2.4. Total phenols and total amino acids concentrations at 70 days after transplanting:

Table (4) revealed that the highest amino acids and total phenols concentrations were with yeast extract at 30 and 15 ml/L. and yeast extract at 15 mg/L compared with those of control plants.

Wahid and Ghazanfar (2006) reported that phenolics, including flavonoids, anthocyanin, lignins,...etc, are the most important class of secondary metabolites in plants and play a variety of roles including tolerance to a biotic stresses. On the other hand the role of amino acids in a biotic stress resistance was reported by Singh (1999). he reported that this class of molecules includes certain amino acids (notably proline), quaternary ammonium compounds. These compounds are thought to play a pivotal role in plant cytoplasmic osmotic adjustment in response to osmotic stresses.

2.5. Endogenous Phytohormones:

Data in Table (5) show the Phytohormones those promote growth aspects growth promoters, (auxins, gibberellin and cytokinin) were highly increased with different assigned treatments. Here the treatment of yeast extract at 30 ml/L gave the highest value activity of promoting phytohormones level, where the increment reached more than three times of control value

Increment of endogenous hormones in tomato plants obtained in the present study could be interpret both of the obtained modifications in

Table (5): Effect of yeast extract, amino acids and citric acid on antioxidant enzymatic activity and endogenous phytohormones ($\mu\text{g/g}$ fresh weight) in tomato (*Lycopersicon esculentum*, Mill.) leaves at 70 days after transplanting during 2007 late summer season.

Characters Treatments	Antioxidant enzymatic activity			Endogenous Phytohormones $\mu\text{g/g}$ fresh weight					
	mg/g fresh weight			Promoters				Inhibitors	Promoters/ Inhibitors
	Peroxidase	Catalase	Superoxide dismutase	Auxins (IAA)	Gibberellins (GA ₃)	Cytokinins	Total	Abscisic acid (ABA)	
1. Yeast extract at 15 ml/L.	187.00	65.00	213.00	68.74	52.32	97.32	218.38	1.56	139.99
2. Yeast extract at 30 ml/L.	163.00	33.00	198.00	81.53	63.59	101.79	246.91	1.48	166.83
3. Amino acids at 1.5 ml/L.	193.00	104.00	197.00						
4. Amino acids at 3 ml/L.	183.00	83.00	266.00	51.67	30.32	76.26	158.25	3.22	49.15
5. Citric acid at 2.5g/L.	22.00	112.00	203.00	62.51	26.43	82.71	171.65	3.15	54.49
6. Citric acid at 5 g/L.	211.00	55.00	217.00						
7. Control	295.00	113.00	254.00	33.84	16.67	35.13	85.64	4.89	17.51

different studied histological features (Tables, 6& 7) and yield (Tables, 2& 3). For example, increasing cytokinins could be in favor of increasing of sink organs (i.e., fruits) ability to accumulate and storage more assimilates. Once again, yeast treatments showed the highest value of cytokinins, IAA and GA₃ in tomato leaves. Yeast is a natural source of cytokinins and has stimulatory effects on plants (Barnett *et al.*, 1990). Yeast has also higher contents of different nutrients, higher percentage of proteins and higher value of vitamins as reported by El-Mogy *et al.*, (1998). This may explain the increase of cytokinins and other promoting hormones in response to yeast application.

Moreover, the proportions of total promoters to the inhibitor abscisic acid Table (5) was increased with the different assigned treatments compared with the control and reached its maximum value with yeast extract at 30 ml/L. In this respect, these results being of great interest for interpreting each of the obtained vigorous growth and the great fruit yield of tomato plant attained in the present study.

As regard to the advantageous of yeast preparation could be due to its essential bioconstituents, i.e. carbohydrate, protein, GAs, IAA, cytokinins and vitamins as well as mineral content El-Tohamy and El-Greadly (2007). Abscisic acid (ABA) and ethylene (C₂H₄), as stress hormones, are involved in the regulation of many physiological properties by acting as signal molecules. Also, different environmental stresses, including high temperature, result in increased levels of ABA (Larkindalr and Haung, 2005).

2.6. Enzymatic antioxidants activity:

Plants posses antioxidant system in the form of enzymes such as superoxide dismutase (SOD), catalase, (CAT) peroxidase (PX) and metabolites, as ascorbic acid, glutathione, and α -tocopherol, carotenoid, flavanoids.....ect, these antioxidant enzymes and metabolites are reported to increase under various environmental stress.

In this respect, data in Table (5) clearly show that different applied treatments induced reductions in the peroxidase, catalase and superoxide dismutase actives as compared with those of the untreated plants in tomato leave at 70 days after transplanting during the lat summer season of 2007. These reductions in determined enzymatic antioxidants activity with different applied treatments compared with control one might be due to their direct scavenging function against the toxic free radicals (induced by heat stress) and / or their promotional effects on synthesis of internal protective antioxidants, i.e., total sugars, total phenols, total amino acids and carotenoids as well as they induce an potent biosynthesis case due to the higher photosynthetic pigments content (protection of chlorophyll's and chloroplasts against stress degradable/senescence effects), thereby higher carbohydrates accumulation and higher minerals (N, P, K, Ca, Mg, Fe, Zn and Mn) constituents vs. growth as mentioned before. Hence, the obtained

Table (6): Effect of yeast extract, amino acids and citric acid on the mean counts and measurements of certain histological features of main tomato (*Lycopersicon esculentum*, Mill.) stem micron at 70 days after transplanting as affected by different applied treatments.

Histological characteristics (micron) Treatments	Stem diameter	Cuticle layer thickness	Epidermal thickness	Thickness of collenchyma layers	Thickness of parenchyma layers	outer phloem thickness	Cambium al region thickness	Xylem thickness	Number of xylem rows/Vascular cylinder	No. of xylem vessels / row	diameter of the widest xylem vessel in V. cylinder	Thickness of inner phloem	Parenchymatous pith thickness	Trichome length in the epidermal layer
1. Yeast extract at 15 ml/L.	8090.30	16.30	43.20	224.10	106.30	136.35	64.80	808.65	88.00	8.80	113.29	189.45	3069.00	201.15
2. Yeast extract at 30 ml/L.	7248.60	17.10	40.95	214.65	155.25	103.95	75.80	761.40	86.00	18.00	105.00	185.40	4140.00	175.50
3. Amino acids at 1.5 ml/L.	5274.00	20.25	41.40	165.15	80.55	81.65	51.75	652.50	89.00	6.50	108.00	137.25	2988.90	223.65
4. Amino acids at 3 ml/L.	5683.60	17.10	39.15	142.20	81.90	111.15	47.25	783.00	76.00	11.00	109.20	190.80	2758.50	214.65
5. Citric acid at 2.5g/L.	6869.70	16.20	31.50	165.15	88.65	113.40	84.60	1003.05	111.00	11.83	128.70	186.30	3492.00	205.65
6. Citric acid at 5 g/L.	5494.60	11.70	39.15	178.65	99.00	68.40	63.00	887.85	103.00	11.75	106.43	157.50	2640.00	213.53
7. Control	6977.25	16.65	48.60	347.40	180.45	154.80	78.30	956.70	70.00	8.80	153.82	216.90	3294.45	290.70

Table (7): Effect of yeast extract, amino acids and citric acid on the mean counts and measurements of certain histological features of tomato (*Lycopersicon esculentum*, Mill.) leaf at 70 days after transplanting as affected by different applied treatments.

Histological characteristic (micron)	Treatments																	
	Thickness of upper epidermis cuticle layer	Thickness of Lower epidermis cuticle layer	Upper epidermis thickness	Lower epidermis thickness	Palisade tissue thickness	Spongy tissue thickness	Thickness of blade	Thickness of collenchyma layers below the upper epidermis at midrib	Thickness of collenchyma layers above the lower epidermis at midrib	Thickness of upper most phloem in the vascular bundle	Thickness of lower most phloem in the vascular bundle	Thickness of xylem tissue	Number of xylem vessels in the vascular bundle	thickness of widest xylem vessel in the vascular bundle	Length of midrib vascular bundle	Width of midrib vascular bundle	Thickness of leaf midrib	Length of Trichome in the epidermal layer
1. Yeast extract at 15 ml/L.	15.75	9.90	32.17	21.60	127.80	162.45	369.67	232.20	156.60	135.00	137.70	211.95	128.00	37.80	572.40	822.15	1708.20	231.75
2. Yeast extract at 30 ml/L.	9.90	8.10	33.30	19.80	158.60	165.60	393.30	236.25	132.30	124.20	165.15	218.25	124.95	41.85	508.50	781.20	1760.85	174.60
3. Amino acids at 1.5 ml/L.	13.50	11.25	27.90	19.35	120.60	133.20	325.80	226.35	175.50	72.00	81.00	171.45	88.82	37.35	356.85	648.00	1197.00	288.00
4. Amino acids at 3 ml/L.	14.40	11.70	34.20	24.30	151.92	144.45	380.97	251.55	102.60	166.50	96.75	249.30	199.41	43.20	487.35	980.55	1687.95	228.15
5. Citric acid at 2.5g/L.	14.85	11.70	32.85	27.85	158.40	197.40	440.05	196.20	144.00	71.10	101.70	178.20	88.00	29.25	373.95	1309.50	816.30	285.75
6. Citric acid at 5 g/L.	13.50	10.35	45.00	37.80	100.35	223.87	430.87	261.00	86.40	121.50	104.85	264.70	192.00	37.20	515.70	1120.50	1917.00	228.90
7. Control	13.95	10.80	29.70	20.70	109.80	137.25	322.20	210.60	129.30	167.40	83.25	203.85	133.00	34.43	356.40	834.30	1310.85	169.20

results in the present study confirmed and coincided such functions and roles of antioxidants. Xu *et al.*, (2006) reported that in addition to tissue dehydration, heat stress may induce oxidative stress which reactive oxygen species (ROS), such as the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH), are by-products of normal cell metabolism that can damage many cellular components, including lipids, proteins and nucleic acids. The conditions leading to damage caused by ROS are referred to as oxidative stress, which can lead to an inhibition of photosynthesis and respiration, and there for, plant growth. Plants have evolved well-developed defense mechanisms against these ROS, involving enzymatic and non-enzymatic scavenging systems, Under unstressed conditions, however, including heat stress, the defense system can be overwhelmed, and is then unable to remove the additional ROS with increased enzymatic or non-enzymatic antioxidant processes. While superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) are key enzymatic antioxidants. SOD catalyses the first step in the scavenging system of ROS by the dismutation of O_2^- by CAT or APX. GR can also remove H_2O_2 via the ascorbate-glutathione cycle.

Also, increasing such bioconstituents reveal the stimulative effect of these treatments to enhance, the internal metabolically protective status by their direct scavenging functions against the toxic free radicals (induced by heat stress) under their promotional effect on synthesis of natural protective antioxidants, i.e. total phenols and carotenoids as well as they induce an potent biosynthesis case due to the higher photosynthetic pigments content (protection of chlorophyll's and chloroplasts against stress degradable/ senescence effects). Thereby, higher carbohydrates accumulation and content as well as higher minerals (N, P and K) content. The strong positive correlations of such constituents vs. growth and fruit yield confirmed and coincided such functions and roles of antioxidants Fathy *et al.*, (2003).

Such results are connected with those reported by El-Mogy *et al.*, (1998), El-Tohamy and El-Greadly (2007) for Yeast ; Abou Dahab and Abd -El-Aziz (2006) for amino acids and Fathy *et al.*, (2003) for citric acid.

3. Anatomical study:

3.1. Effect of different applied treatments on the stem anatomy:

Table (6) and Fig. (1) show that different applied treatments increased the stem diameter compared with control. This increase reached its maximum values with yeast extract at 30 ml/L gave 7248.60 μ compared with the stem diameter was 6977.25 μ of the control .

Also, it could be noticed that increase of the stem diameter of stem were reversed upon different tissues comprising the whole section . Since,

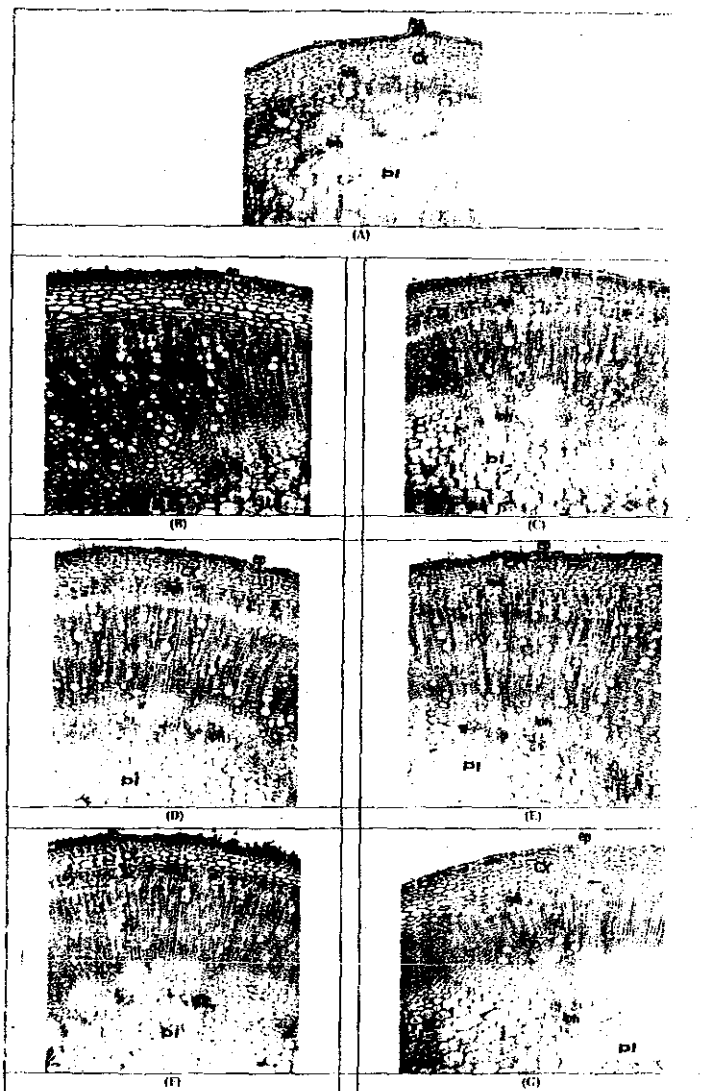


Fig. (1): Transverse sections (X = 25) through 5th internode of the main stem of tomato plants at 70 days after transplanting as affected by different applied treatments.

Where: (A): Control (B): Yeast extract at 15 ml/L, (C): Yeast extract at 30 ml/L, (D): Amino acids at 1.5 ml/L
 (E): Amino acids at 3 ml/L. (F): Citric acid at 2.5 g/L and (G): Citric acid at 5 g/L
 ep= Epidermis cx= Cortex oph= outer phloem tissue
 iph=inner phloem tissue xy= Xylem tissue pi= pith

thickness of each cuticle layer, epidermis, cortex (collenchyma and parenchyma tissues) and pith parenchyma layers, as well as the dimensions of vascular bundles. Moreover, thickness of outer & inner phloem tissues, of cambial region and of xylem tissue, number of xylem vessels /vascular bundle and diameter of the widest xylem vessel were greatly increased compared with the control. Also, yeast extract at 30 ml/L. and amino acids at 1.5 ml/L. treatments were more pronounced in this respect.

In this respect, other studies reported nearly similar findings of these are Ismaeil and Bakry (2005), using yeast extract on papaya. In general, the stimulatory effects of applied treatments upon the anatomy features of treated plants could be attributed to the effect upon cambium activity. Increment of cambium activity could mainly attributed to the increase of endogenous hormones level especially cytokinins and auxins, (Ismaeil and Bakry, 2005) as well as the findings of the present study.

3.2. Effect of different applied treatments on tomato leaf anatomy:

Data in Table (7) and Fig. (2) clearly indicate the effect of different applied treatments upon alleviating the adverse effects of the high temperature stress on different anatomical features of tomato leaves. In this respect, most of the studied features of leaf anatomy were increased with different applied treatments. Among these anatomical features were the most important ones, i.e., thickness of midrib, length & width of vascular bundle, phloem & xylem tissues and number of xylem vessels in vascular bundle as well as the leaf blade thickness.

With regard to the blade thickness, it was increased with different used treatments to reach its maximum value (440.05 μ) with citric acid at 2.5 g/L. and (393.30 μ) with yeast extract at 30 ml/L treatment. That represent of the control value (322.20 μ). Also the thickness of each of upper and lower epidermis, were also increased with all applied treatments. Also it could be noticed that increase ratio was higher of upper epidermis than that of the lower one.

For mesophyll tissue, thickness of both spongy and palisade tissues were increased with different applied treatments. Here, spongy tissue thickness was 137.25 micron in the control but increased to reach 223.87micron with citric acid at 5 g/L, which was the more effective treatments in the same order. Also, palisade tissue thickness was 109.80 micron of control but increased to reach 158.40 and 156.60 micron with citric acid at 2.5g/L., yeast extract at 30 ml/L. which were the more effective treatments in the same order. Of interest, to note that mesophyll increase belong to that increase of each of palisade and spongy tissue thickness. Since, the two components were increased with all applied treatments but reached their maximum as other traits with yeast extract at 30 ml/L treatment.

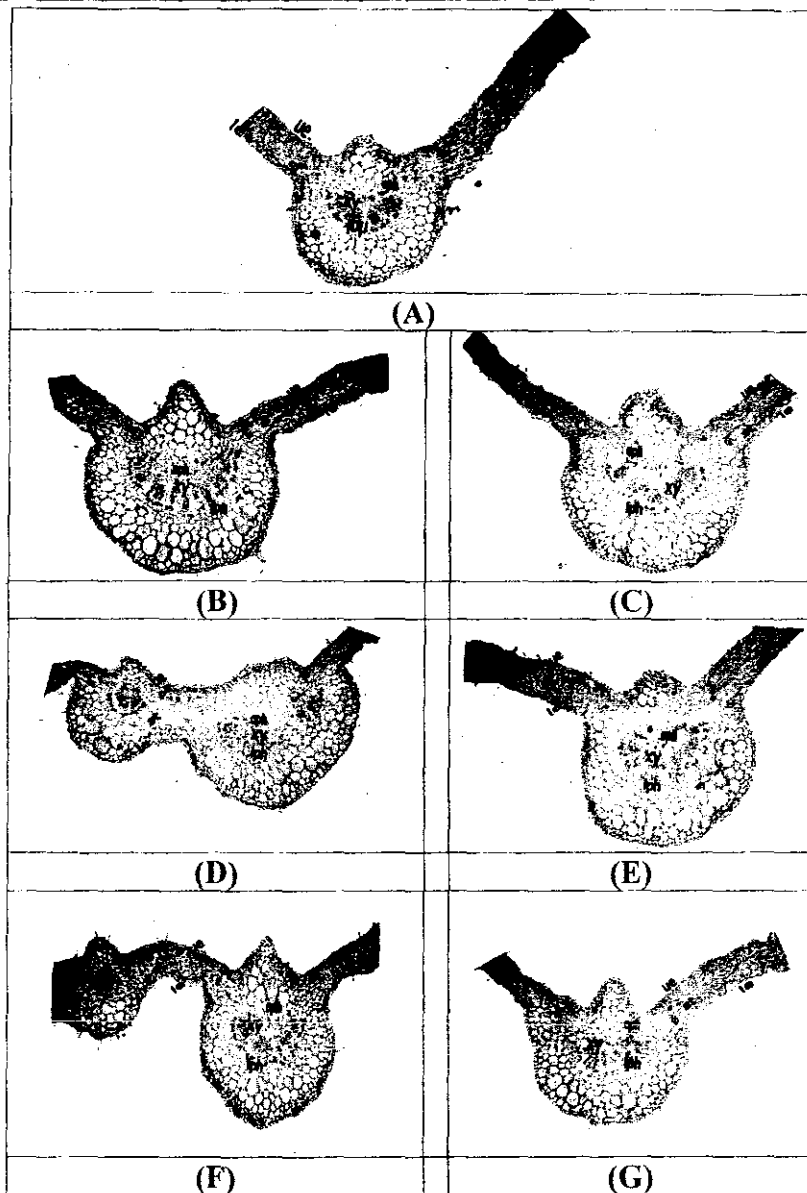


Fig. (2): Transverse sections (X = 40) through 4th apical leaf of tomato plants at 70 days after transplanting as affected by different applied treatments.

Where: (A): Control (B): Yeast extract at 15 ml/L, (C): Yeast extract at 30 ml/L, (D): Amino acids at 1.5 ml/L
 (E): Amino acids at 3 ml/L. (F): Citric acid at 2.5 g/L and (G): Citric acid at 5 g/L
 oph= outer phloem tissue xy= Xylem tissue iph=inner phloem tissue
 ue= Upper epidermis pt= Palisade tissue st= Spongy tissue
 le= Lower epidermis

With regard to midrib anatomical features, could be noticed that increment in the midrib thickness with different applied treatments attributed to the increase in many of its histological features such as thickness of both uppermost and lower most collenchyma tissues, lower most parenchyma tissue and dimensions of main vascular bundle as well as thickness of upper most & lower most phloem tissues, xylem tissue and also number and diameter of xylem vessels in the main vascular bundle. This increases were more obvious with the yeast extract at 30ml/L. The above mentioned results specially increment of the conductive tissues (xylem & phloem) are also of great importance because they could be also involved in the interpretation about why vigorous growth and high yielded fruits were existed with different applied treatments specially with yeast extract at 30 ml/L.

In general, these positive alterations in leaf anatomy of tomato plants treated with applied treatments led to vigorous growth and enhancement of flowering and fruit setting of treated plants. That as well mentioned afterwards reversed upon high increases in the final fruit yield. Besides, yield increases with different applied treatments through doing alterations in the anatomical features of treated plants was also reported by Ismaeil and Bakry (2005).

Also, Añon *et al.*, (2004) reported that although limited details are available, anatomical changes under high ambient temperatures are generally similar to those under drought stress. At the whole plant level, there is a general tendency of reduced cell size, curtailed water loss and increased trichomatous densities and greater xylem vessels of both root and shoot.

Finally, it could be concluded with spraying tomato plant (under late summer/ heat stress conditions), 5 times with yeast extract at 30 ml/L for maximizing fruit yield.

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تأثير مستخلص الخميرة والأحماض الأمينية وحمض الستريك على الخصائص الفسيوتشرحية والإنتاجية لنباتات الطماطم النامية في العروة الصيفي المتأخرة

سعيد على الدسوقي^(١)، فاتن حسن محمود إسماعيل^(١)، أحمد لطفى ونس^(٢)،
السعيد لطفى السيد فتحى^(٣)، محمد محمد محمود عبد العال^(١)

^(١) قسم النبات الزراعي - كلية الزراعة - جامعة بنها - قسم النبات الزراعي

^(٢) كلية الزراعة - جامعة المنصورة - فرع دمياط

^(٣) قسم الخضار - معهد بحوث البساتين - مركز البحوث الزراعية

الملخص العربي

أجريت تجربتان حقليتان بمحطة بحوث الخضار التابعة لقسم البساتين - كلية الزراعة - جامعة بنها في العروة الصيفية المتأخرة خلال عامي ٢٠٠٦ ، ٢٠٠٧ علي نبات الطماطم صنف سوبر ستارين بي بهدف دراسة تأثير استخدام بعض المواد الطبيعية (كمضادات للأكسدة) علي بعض الصفات الفسيولوجية والتشريحية والتركيب الكيماوي وأيضاً علي التزهير و المحصول ومكوناته وجودة الثمار الناتجة لنباتات الطماطم المعاملة. وكانت المعاملات كالاتي:

١. مستخلص الخميرة بتركيزي ١٥ ، ٣٠ مل/لتر

٢. أحماض أمينية بتركيز ١,٥ ، ٣ مل/لتر

٣. حمض الستريك بتركيزي ٢,٥ ، ٥ جم/لتر

٤. المعاملة بالماء المقطر (كنترول)

ويمكن إيجاز أهم النتائج المتحصل عليها كما يلي :

١- معاملة مستخلص الخميرة ٣٠ مل/لتر أعلى قيم لعدد الأزهار و أعلى نسبة عقد الثمار، حيوية حبوب اللقاح وكذلك زيادة العدد الكلي للثمار بالنسبة للنبات و تحسين صفات ثمار نباتات الطماطم الطبيعية ممثلة في زيادة متوسط وزن الثمرة ، طول الثمرة ، قطر الثمرة وشكل الثمرة و كذلك أدت إلي تحسين القيمة الغذائية لثمار الطماطم حيث أدت إلى زيادة محتواها من فيتامين ج و محتواها من بعض العناصر المعدنية مقارنة بالكنترول.

- ٢- أدت جميع المعاملات المختلفة المستخدمة إلى زيادة صبغات التمثيل الضوئي ، تركيز عناصر النيتروجين ، الفوسفور ، البوتاسيوم، الماغنسيوم وكذلك عنصر الحديد و السكريات الكلية والكربوهيدرات الكلية وكذلك كل من الأحماض الأمينية الكلية والفينولات الكلية كما أدت أيضا إلى زيادة البروتين الكلى في أوراق الطماطم بعد ٧٠ يوم من الشتل خلال موسم ٢٠٠٧م و التركيز الهرموني الداخلى للمستيوكيتيات والجبريلينات والأوكسينات بينما أدت إلى نقص واضح في المثبطات الداخلية مثل حمض الأبوسيك و تركيز عناصر النيتروجين ، الفوسفور ، البوتاسيوم، الكالسيوم مقدره بالمعجم/جم مادة جافة وكذلك عنصر الحديد مقدر كجزء/مليون في الثمار.
- ٣- أدت المعاملات المستخدمة إلى نقص النشاط الإنزيمي لمضادات الأكسدة الإنزيمية التي تم تقديرها وهي الكتاليز والبيروكسيداز والسوبرأوكسيد ديسميوتاز مقارنة بالكنترول وهذا النقص في النشاط الإنزيمي قد يرجع إلى دور هذه المعاملات كمضادات أكسدة في إزالة أو التخلص من الشقوق الحرة السامة.
- ٤- أظهرت الدراسات التشريحية أن النمو القوي لنباتات الطماطم نتيجة المعاملات المختلفة المستخدمة كان مصحوبا بتغيرات واضحة في العديد من الصفات التشريحية للساق و الأوراق. حيث أدت جميع المعاملات المستخدمة إلى زيادة سمك الساق والأنسجة المكونة له مثل البشرة والقشرة والنخاع البارانشيمي هذا بالإضافة إلى زيادة سمك العرق الوسطي والنصل وكذلك البشرة العليا والبشرة السفلى والنسيجين العمادي والأسفنجي في الأوراق. وعلاوة على ذلك فقد زادت أبعاد الحزم الوعائية وسمك نسيجي اللحاء والخشب وكذلك عدد أوعية الخشب بالحزمة الوعائية وهذا يؤكد أهمية المساحة المقطعية لنسيجي اللحاء والخشب والذي صاحبه تخليق كمية أكبر من نواتج التمثيل وامتصاص أكثر للعناصر المعدنية مما انعكس على تحسين النمو والإنتاجية للنباتات المعاملة.
- ٥- في ضوء هذه الدراسة يمكن التوصية برش نباتات الطماطم تحت ظروف إجهاد الحرارة المرتفعة (خلال العروة الصيفية المتأخرة) بمستخلص الخميرة بتركيز ٣٠ مل/لتر ٥ مرات للحصول على أعلى إنتاجية.