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Diverse responses for some growth biostimulants on some morphological, physiological, chemical, anatomical and yield characteristics of tomato plant (*Lycopersicon esculentum* Mill)

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Abstract

Two field experiments were carried at the Experimental Farm Station of The Faculty of Agriculture, Benha University, Qlubia governorate and Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University (Assiut Branch), Assiut governorate, Egypt, during two successive summer seasons of 2016 and 2017.The aim of this work was to study the effect of foliar application with Arginine at 25, 50 mgl^{-1} and Tryptophan at 50, 100 mgl^{-1} as well as Milagrow at 50 and 100 mgl^{-1} treatments comparing to the control on morphological, physiological, chemical, anatomical, flowering and yield characteristics of tomato plant. The experiment was performed as a randomized complete block design with four replicates. Different vegetative growth characteristics of plant samples were taken i.e., plant length, stem diameter, number of branches plant^{-1}, number of leaves plant^{-1}, stem fresh weight plant^{-1}, leaves fresh weight plant^{-1}, stem dry weight plant^{-1}, leaves dry weight plant^{-1} and total leaf area plant^{-1}. Photosynthetic pigments in the leaves i.e., Chlorophyll A, B, A+B and Carotenoids mg g^{-1} F.Wt., phytohormones i.e., Gibberellins, Auxins, Cytokinins and total promoters as well as Salicylic acid and Abscisic acid content. Anatomical characteristics of tomato plant leaf i.e., thickness of upper epidermis tissue, lower epidermis, palisade tissue, spongy tissue, blade, phloem tissue, xylem tissue, No. of xylem rows, widest of M. xylem vessel, length of vascular bundle, width of vascular bundle and thickness of leaf midrib. Anatomical stem characteristics i.e., thickness of epidermis tissue, collenchyma layers, parenchyma layers, phloem tissue, cambial tissue, xylem tissue, No. of xylem rows vascular cylinder^{-1}, No. of xylem vessels row^{-1}, widest of M. xylem vessel, thickness of parenchymamatous pith and stem diameter. From these results we can conclude that, the beast treatment was Milagrow at 50 mgl^{-1} flowed by Arginine at 50 mgl^{-1} comparing with the control and other applied biostimulant treatments during the both seasons. On the other side, the treatment with Tryptophan, recorded low values, especially at 50 mgl^{-1} comparing with the other biostimulants. At the same time Milagro at 50 mgl^{-1} which recorded the highest values for early yield (g) plant^{-1} (731.62), total fruits No. plant^{-1} (29.74), fruit setting % (37.19), total yield plant^{-1} (1.94 kg), fruit fresh weight fruit^{-1} (67.38 g) and fruit dry weight fruit^{-1} (3.14g). The highest values for nitrogen, crude protein, total carbohydrates, total soluble solids and Vitamin C mg100^{-1} cm3 juice was recorded with Milagrow at 50 mgl^{-1}. On the contrast, the same treatment was recorded low values for phosphorus, potassium and total acidity comparing with the other treatments during both seasons. The negative correlation was found among nitrogen, crude protein, total carbohydrates, total soluble solid and vitamin C mg 100^{-1} cm^{3} juice with phosphorus, potassium and total acidity. From these results can conclude that, the beast treatment was Milagrow at 50 mgl^{-1} flowed by Arginine at 50 mgl^{-1} comparing with the control during the both seasons. On the other side, the treatment with Tryptophan, recorded low values, especially at 50 mgl^{-1} comparing with the other applied biostimulants. Finally, the leaf and stem anatomical characters indicated that Milagrow was the beast treatment for enhancement the diameter of xylem vessel in the stem and No. of xylem rows in the leaf and stem especially at 50 mgl^{-1} as well as increasing the thickness of leaf lamina and midrib especially at 100 mgl^{-1}, subsequently increasing the mineral elements transport and photosynthetic rate. Moreover, increasing yield and may be caused the tolerance to an adverse conditions especially high temperature for tomato genotypes during summer season.

Keywords: tomato, arginine, tryptophan, milagrow, anatomical characteristics, photosynthetic pigments, yield and quality.

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1. Introduction

Tomato (*Lycopersicon esculentum* Mill) is one of the most popular members of Solanaceae family and considered a major vegetable crop in several parts of the world including Egypt. Tomato is used in many forms i.e., fresh salad, cooked food, ketchup, paste and so on. It have essential roles in human nutrition since, it is a rich source of lycopene, minerals and organic acids as well as vitamins i.e., ascorbic acid and β-carotene which are considered as antioxidants and promoting good health (Wilcox *et al.,* 2003). Lack of tolerance in most tomato cultivars for high temperature is a major limitation for growing in many regions, which the temperature during part of the growing season, even for short period, reaches or higher than 38°C. High temperature has an adversely effects on tomato vegetative and reproductive growth as well as reducing yield and fruit quality characteristics. Heat stress affects many biochemical and physiological processes finally, leading to non-uniform growth and reduction of tomato yield or even completely tomato cropping failure (Adil *et al.,* 2004, Saeed *et al.,* 2007, Pressman *et al.,* 2002). In order to address food insecurity for increasing population at the national level, it is necessary to maximize yield per unit area per unit farm input. Plant bio regulators represent one area of research for increasing crop productivity as well as water and fertilizers use efficiency and then profitability (Pelt and Popham, 2006). Using the naturally occurring substances such amino acids for enhancing plant growth characteristics was concluded by Abou Dahab and Abd El-Aziz (2006). These growth naturally occurring substances derived from plant and seaweed have not detrimental effects on human being and animals as well as the environment. Using amino acids as a precursor for plant growth promoters is an approach for minimizing the stress effects on plant growth and productivity. L-Tryptophan is a common precursor of plant hormone (auxin) that affects many plant physiological processes after uptake directly or after transforming to auxin (Rai, 2002). L-tryptophan acts as an osmolyte, regulator of ion transport, modulates opening of stomata. Moreover, pathway of tryptophan is playing a defensive role in plant (Hussein *et al.,* 2014). El-Badawy and Abd El-Aal (2013) indicated that foliar spray of mango plant with tryptophan enhanced most of the morphological, physiological and leaf anatomical features. Abd El-Aal and Eid (2018) found that foliar application with amino acids at 4 ml^{-1} treatment increased the vegetative growth, physiological and yield characteristics as well as leaf and stem anatomical features of soybean plant. Arginine is an essential amino acid and considered as the polyamines precursor, agmatine and proline as well as the molecules of cell signaling glutamine and nitric oxide (Chen *et al.*, 2004, Liu *et al.*, 2006) which produced by decarboxylation of arginine via arginine decarboxylase to form putrescine (Bocherueu, 1999, Evans and Malmberg, 1989). Polyamines and arginine involved in the cell cycle control, cell division, morphogenesis and plant hormone mediated process and plant senescence control as well as in responses of plants to different stress factors (Walters, 2000). The application of arginine which is one of the essential amino acids increases and promotes the growth and fresh as well as dry weight, some endogenous growth regulators, chlorophylls a, b and carotenoids in bean plant (Nassar *et al*., 2003) and wheat (El-Bassiouny *et al*., 2008). Moreover, many researchers have showed the arginine positive role for alleviating the adverse effects occurring as a result for exposing plant to stress (Hassanein *et al*., 2008, Khalil *et al*., 2009). It has been reported that arginine have several roles in higher plants growth responses (Nasibi *et al*., 2011, Song *et al*., 1999, Zhang *et al*., 2011, Zeid, 2009). Exogenous positive effects on growth and development in stress conditions have been found NO (Liu *et al*., 2011, Neill *et al*., 2003), PAs (He *et al*., 2002) and proline (Gioseffi *et al*., 2012, Patton *et al*., 2007)**.** Milagrow (Brassinosteroids, BRs) has been found for stimulating cell division and elongation, differentiation of flowering bud, carbohydrate assimilation and activity of ATP, subsequently improving vegetative growth as well as enhancing physiological status then directing plant for earlier harvest and increasing fruit yield and quality features (Gabr *et al*., 2011, Gomes *et al*., 2006, Symons *et al*., 2006). Salazar *et al*. (2016) reported that Brassinosteroid treatments increased marketable fruit yield of strawberry plant. Milagrow is natural growth promoter, it is extracted from cabbage flowers pollen grain. It promotes growth and flower buds formation, resist dropping of flowers and fruits, increases fruits setting percentage, subsequently increasing of yield and quality. Milagrow has combined effects of auxins, gibberellins, cytokinin, hydrogen cyanamid and ethylene. Its chemical constituents are 0.2% brassinoliods (BRs), 10% potassium, 20% phosphorus and 3% boron (Attia *et al.*, 2011, Seadh *et al.*, 2012). Attia *et al.* (2011) mentioned that foliar application with Milagrow significantly recorded the highest values of plant height, number of branches plant^{-1} as well as yielded petal fed^{-1} (1 feddan (fed) = 4200 m² = 0.42 hectares = 1.038 acres) of safflower plant. Seadh *et al.* (2012) reported that the highest values of capitula number plant^{-1}, weight of 100 seed of safflower plant. At high temperature, the flower reproductive part is adversely affected. Elongation of stigma tube, pollen poor germination, poor growth of pollen tube and carbohydrate stress are the main reasons to poor fruit set in tomato plant at high temperature. Vollenweider and Gunthardt-Goerg (2005) and Sato *et al.* (2006) stated that under high temperatures, fruit setting of tomato plant failed due to sugar metabolism disruption and transport during the narrow window of male reproductive development. General environmental changes, especially global warming in Egypt, have adverse effects on crop productivity. So, the main objectives of this study is to investigate if there are positive effects of the growth biostimulant treatments i.e., Arginine, Tryptophan and Milagrow on improving morphological, physiological, anatomical, flowering and yield characteristics as well as their roles in the tolerance to the adverse effects of high temperature on tomato plant during summer season.

2. Materials and methods

The present study was carried out in the field at The Experimental Farm of Faculty of Agriculture, Moshtohor, Qalubia Governorate, and Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University (Assiut Branch), Assiut governorate, Egypt during the two growing successive summer seasons of 2016 and 2017. The experiment was performed as a randomized complete block design with four replicates. In both seasons the normal agricultural practices of growing tomato plant including equal amounts of fertilizer and irrigation water were applied. The applied treatments were used as foliar application at different concentrations as follows: Control (Tap water) 0.0, Arginine at 25 and 50 mgl^{-1}, Tryptophan at 50 and 100 mgl^{-1}, Milagrow (Brassinosteroids, BRs) is a class of poly hydroxyl steroids that have been recognized as a class of plant hormones Mitchell *et al.*, (1970) at 50 and 100 mgl^{-1}. Plants of each treatment were sprayed three times at 40, 55 and 70 DAS throughout the two summer seasons of 2016 and 2017.

2.1 Sampling and collecting data

Different vegetative growth characteristics of plant samples were taken at 65 DAS during the experimental period of tomato plant (cv.UC82). Three plants from each treatment were randomly taken for different measurements. The samples of these organs were dried in the oven at 70 C° for 48 hours till constant weight. The dried samples of different organs were weighted for dry weight estimation then kept for chemical analysis determinations.

2.2 Morphological characteristics of vegetative growth

The following vegetative growth characteristics were estimated i.e., plant length, stem diameter (cm), number of branches $plant^{-1}$, number of leaves $plant^{-1}$, stem fresh weight $plant^{-1}$, leaves fresh weight $plant^{-1}$, stem dray weight $plant^{-1}$, leaves dry weight $plant^{-1}$ (g) and total leaf area (cm^2) $plant^{-1}$.

2.3 Physiological characteristics

Chemical analysis was carried out at 65 DAS during seasons of 2016 and 2017.

2.3.1 Determination of photosynthetic pigments in the plant leaves

Determination of photosynthetic pigments in the plant leaves i.e., chlorophyll A, B, A+B and carotenoids mg g^{-1} F.Wt. calorimetrically determined in the fresh leaves according to Wettestein (1957).

2.3.2 Endogenous phytohormones

Endogenous phytohormones (Promoters) i.e., Gibberellins, Auxins, cytokinins and total promoters as well as the salicylic acid were quantitatively determined in tomato shoot during 2017 season using High Performance Liquid Chromatography (HPLC) according to

Koshioka *et al*. (1983) for auxins (IAA), gibberellins, salicylic Acid and abscisic acid (ABA) while cytokinins were determined according to Nicander *et al*. (1993).

2.4 Anatomical characteristics

The effect of foliar application with Arginine at 25 and 50 mgl^{-1}, Tryptophan at 50 and 100 mgl^{-1} and Milagrow at 50 and 100 mgl^{-1} treatments comparing with the control (distilled water) on leaflet and stem anatomical characters of tomato (cv. UC82) plants was studied. Specimens of tomato (cv. UC82) terminal leaflets and stems were taken from the 4th apical internode of the main stem and its corresponding leaf of treated plants and those of the control at 65 DAS during season of 2017 only. These 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 12 microns in thickness for leaflets and stems (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^{-1} 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^{-1} were calculated. Anatomical characteristics of the treated plants compared with the control based on transverse sections of the leaflets and stems were studied.

2.4.1 Anatomical of leaf

Anatomical of leaf characters i.e., thickness of upper epidermis tissue, lower epidermis, palisade tissue, spongy tissue, blade, phloem tissue, xylem tissue, No. of xylem rows, thickness of widest M. xylem vessel, length of vascular bundle, width of vascular bundle and thickness of leaf midrib.

2.4.2 Anatomical of stem

Anatomical of stem characters i.e., thickness of epidermis, collenchyma layers, parenchyma layers, phloem tissue, cambial tissue, xylem tissue, number of xylem rows vascular cylinder^{-1}, number of xylem vessels row^{-1}, diameter of widest M. xylem vessel, thickness of parenchy-mamatous pith and stem diameter.

2.5 The flowering and yield parameters

2.5.1 Flowering and yield

Flowering and yield i.e., earliness of flower anthesis, number of flower plant^{-1}, pollen grains fertility percentage, early yield number plant^{-1}, early yield plant^{-1}, total fruits number plant^{-1}, fruit setting percentage, abscission percentage, total yield plant^{-1}, fruit fresh weight fruit^{-1} and Fruit dry weight fruit^{-1}.

2.5.2 Fruit chemical compositions

Fruit chemical compositions i.e., nitrogen, phosphorus, potassium, crude protein, total carbohydrates, total soluble solid, total acidity, vitamin C mg 100^{-1} cm3 juice were determined according to A.O.A.C. (2005).

2.6 Statistical analysis

Data of vegetative growth, yield characteristics and chemical constituents were statistically analyzed and the means were compared using the Least Significant Difference test (L.S.D) at 5% level according to Snedecor and Cochran (1980).

3. Results and Discussion

3.1 Morphological characteristics of vegetative growth

Data in Table (1) show that, all the levels of treatments significantly increased the vegetative growth parameters i.e., plant length, stem diameter, number of branches $plant^{-1}$, number of leaves $plant^{-1}$, stem fresh weight $plant^{-1}$, leaves fresh weight $plant^{-1}$, stem dray weight $plant^{-1}$, leaves dry weight $plant^{-1}$ and total leaf $areaplant^{-1}$. From these results can conclude that, the best treatment was Milagrow at 50 mgl^{-1} flowed by Arginine at 50 mgl^{-1} comparing with the control during both seasons. On the other side, the treatment with tryptophan, recorded low values compared with Milagrow and Arginine treatments, especially at 50 mgl^{-1}. In this respect, the increasing of formed branches on growing plant could be reversed upon many other characteristics such leaves number, leaf area, leaves dry weight as well as increasing the photosynthetic efficiency, flowering and finally the yielded fruits. Foliar application with Milagrow at 50 ppm twice after 30 and 70 DAS significantly recorded the highest values of plant height, number of branches $plant^{-1}$ of safflower (Attia *et al.*, 2011). Milagrow (Brassinosteroids, BRs) has been found to stimulate cell division and elongation, flowering bud differentiation, carbohydrate assimilation and ATP activity, subsequently vegetative growth improving (Gabr *et al.*, 2011, Gomes *et al.*, 2006, Symons *et al.*, 2006). At the same time Arginine treatment significantly increased wheat fresh and dry weight (El-Bassiouny *et al.*, 2008). Moreover, it was reported that arginine has important roles in different environmental stresses, such salinization by scavenging free radical species (Zhang *et al.*, 2011). Arginine application significantly promote growth through increasing fresh as well as dry weight of bean plant (Nassar *et al.*, 2003) and wheat plant (El- Bassiouny *et al.*, 2008).

3.2 Physiological characteristics

3.2.1 Photosynthetic pigments content

The data in Table (2) clearly indicate that, all the applied treatments significantly increased the photosynthetic pigments content in the plant leaves i.e., chlorophyll A, B, A+B and carotenoids mg g^{-1} F.Wt. From these results we can conclude that, the beast treatment was Milagrow at 50 mgl^{-1} comparing with the control and other treatments during both seasons. Moreover, there’re negative correlation between chlorophyll content

A, B and A+B with carotenoids concentration in the leaves with treatments. In general, data in Table (2) not only being a direct results for that vigorous growth obtained in Table (1) but also could be considered an indicator for expectable high yield of fruits. These results are of great interest, 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. The brassinosteroid application in wheat plant stimulated photosynthetic activity expressed by acceleration in fixing of CO2, increasing biosynthesis of protein (Braun and Wild, 1984) and in mustard plant, increased rates of photosynthesis which related to growth and seed production directly (Hayat *et al.*, 2000). It is also evident that brassinosteroids regulate the metabolism of growth through the signals of auxins for promoting cell division, development of root hairs, xylem differentiation, development of pollen, seed setting as well as yield output (Hayat *et al*., 2003). Moreover, Clouse (1996) showed that BRs have been shown for enhancing differentiation of tracheae, stimulate hyperpolarization of membranes as well as activity of ATPase, promoting ethylene bio synthesis, control orientation of microtubule and alter mechanical of cell walls properties. The arginine treatment significantly increased some growth regulators, chlorophylls and carotenoids contents in bean plant (Nassar *et al*., 2003) and wheat plant (El-Bassiouny *et al*., 2008). Tryptophan acts as an osmolyte, regulator of ion transport, modulating of stomata opening and detoxifing of stress harmful effects (Orabi *et al*., 2014, Rai, 2002). Pathway of tryptophan is playing a defensive role in plants (Hussein *et al*., 2014).

Table (1): Morphological characters in tomato plants as affected by some biostimulants at 65 DAS during 2016 and 2017 seasons.

Characteristics / Treatments	Plant length (cm)	Stem diameter (cm)	No. of branches plant^{-1}	No. of leaves plant^{-1}	Stems F.Wt.g plant^{-1}	Leaves F.Wt.g plant^{-1}	Stems D.Wt.g plant^{-1}	Leaves D.Wt.g plant^{-1}	Total leaf area plant^{-1} (cm^2)
Season 2016									
Control	54.28	1.15	5.52	27.61	41.43	83.87	5.76	16.31	2407.08
Arginine at 25 mgl^{-1}	63.69	1.32	6.87	36.50	50.08	97.69	6.09	17.50	2671.52
Arginine at 50 mgl^{-1}	71.49	1.70	7.53	43.43	74.12	161.60	9.45	31.82	3622.86
Tryptophan at 50 mgl^{-1}	59.12	1.30	6.14	34. 76	48.73	94.13	8.90	21.20	2623.01
Tryptophan at 100 mgl^{-1}	68.10	1.36	7.11	38.55	66.37	128.42	7.86	23.60	3230.35
Milagrow at 50 mgl^{-1}	74.26	1.71	8.16	45.66	83.13	165.66	11.45	32.53	4416.29
Milagrow at 100 mgl^{-1}	69.07	1.37	7.09	36.70	59.26	112.08	7.58	22.37	3112.14
L.S.D. 0.05	4.15	0.04	0.82	4.17	6.22	9.16	1.19	2.63	187.26
Season 2017									
Control	60.28	1.30	5.70	33.17	40.22	87.17	6.68	16.77	2592.66
Arginine at 25 mgl^{-1}	62.38	1.64	6.33	33.91	55.07	108.40	8.41	18.17	3176.38
Arginine at 50 mgl^{-1}	68.03	1.57	7.12	41.53	66.69	125.33	9.06	25.80	3433.31
Tryptophan at 50 mgl^{-1}	58.92	1.35	6.31	34. 08	43.17	91.27	7.23	17.70	2672.22
Tryptophan at 100 mgl^{-1}	67.49	1.55	6.47	36.60	58.30	117.57	8.46	22.65	3327.49
Milagrow at 50 mgl^{-1}	70.60	1.62	7.23	42.24	78.68	143.82	10.02	27.22	4055.46
Milagrow at 100 mgl^{-1}	63.71	1.44	6.26	35.09	57.88	98.61	8.34	17.93	2714.00
L.S.D. 0.05	3.27	0.12	0.74	3.52	4.57	6.09	0.98	1.18	205.43

3.2.2 Endogenous phytohormones and salicylic acid contents of tomato shoot

As shown in Table (3) it could be noticed that different applied biostimulants significantly increased the endogenous phytohormones content (Promoters) i.e., gibberellins, auxins, cytokinins and total promoters as well as the salicylic acid in the plant shoot. From these results we can conclude that, the beast treatment was Milagrow at 50 mgl^{-1} which increased the promoters and salicylic acid contents in the shoot, but low values was recorded with the Arginine at 25 mgl^{-1}, in addition to, the highest values with total promoters (637, 27) and cytokines (468.25) µg g^{-1} F.Wt. were recorded with the same dose of Milagrow during the season of study. On the contrast, the lowest value was recorded for the inhibitor (ABA) 0.55 µg g^{-1} F.Wt. Moreover, there're, positive correlation between gibberellins and auxins with tryptophan treatment especially at 100 mgl^{-1}. Increment of endogenous phytohormones in tomato plant obtained in the present study could be interpret both of the obtained modifications in different studied histological features as will be mentioned later Tables (4 and 5) and the growth improvement Table (1) as well as enhancement of bioconstituents Tables (2 and 7) and yield Table (6). For example, increasing cytokinins could be in favor of increasing the formed branches number and that could increase transverse growth on the account of longitudinal one as well as increasing of sink organs (i.e., fruits) ability to accumulate and storage more assimilates. Milagrow is natural growth promoter extracted from cabbage flowers pollen grains. It is growth promoter, it has combined effects of auxin, gibberellin, cytokinin, ethylene as well as hydrogen cyanamid. Its chemical compositions are 0.2% brassinoliods, 20% phosphorus, 10% potassium and 3% boron (Attia *et al.*, 2011, Seadh *et al.*, 2012). Polyamines and their precursor arginine have been implicated as vital modulators in higher plants growth, physiological as well as developmental processes (Glastone and Kaur-Sawhny, 1990). Polyamines are involved in the cell cycle control, cell division and plant hormones mediated process and the plant senescence control as well as in plant response to different stress factors (Walters, 2000).

3.3 Anatomical characteristics

3.3.1 Anatomical characteristics of leaf

Data in Table (4) and Figure (1) indicate that, all biostimulant treatments significantly increased the anatomical of leaf characters i.e., thickness of upper epidermis, lower epidermis tissue, palisade tissue, spongy tissue, blade, phloem tissue, xylem tissue, number of xylem rows, thickness of widest M. xylem vessel, length of vascular bundle, width of vascular bundle and thickness of leaf midrib. From these results can conclude that, the beast treatment was milagrow at 50 mgl^{-1} for thickness of lower epidermis tissue (16µ), palisade tissue (189µ), spongy tissue (230µ), blade (477µ), phloem tissue (241µ) and xylem tissue (592µ) respectively, followed by tryptophan treatment 50 mgl^{-1} which improved the xylem tissue (608µ), number of xylem rows (23), thickness of widest M. xylem vessel (51µ) characteristics respectively, the same results were obtained from tryptophan treatment with length of vascular bundle (727µ), width of vascular bundle (488µ) and thickness of leaf midrib (1793µ) especially at 100 mgl^{-1}, On the other side, the control treatment recorded the lowest value for thickness of leaf midrib (1481µ).

Table (2): Photosynthetic pigments content of tomato leaves as affected by some biostimulants at 65 DAS during 2016 and 2017 seasons.

Treatments \ Characteristics	Chlorophyll mg g^{-1} F.Wt.			Carotenoids mg g^{-1} F.Wt.
	(A)	(B)	(A + B)	
Season 2016				
Control	0.82	0.51	1.33	0.67
Arginine at 25 mgl^{-1}	0.94	0.59	1.53	0.64
Arginine at 50 mgl^{-1}	1.15	0.56	1.71	0.76
Tryptophan at 50 mgl^{-1}	0.91	0.54	1.45	0.87
Tryptophan at 100 mgl^{-1}	1.09	0.49	1.58	0.74
Milagrow at 50 mgl^{-1}	1.21	0.61	1.82	0.81
Milagrow at 100 mgl^{-1}	0.95	0.65	1.60	0.74
L.S.D. 0.05	0.11	0.08	0.21	0.06
Season 2017				
Control	1.03	0.47	1.50	0.70
Arginine at 25 mgl^{-1}	0.83	0.70	1.53	0.68
Arginine at 50 mgl^{-1}	1.10	0.63	1.73	0.83
Tryptophan at 50 mgl^{-1}	0.97	0.54	1.51	0.83
Tryptophan at 100 mgl^{-1}	1.12	0.58	1.70	0.81
Milagrow at 50 mgl^{-1}	1.22	0.59	1.81	0.65
Milagrow at 100 mgl^{-1}	1.08	0.72	1.80	0.76
L.S.D. 0.05	0.04	0.10	0.15	0.03

Table (3): Endogenous phytohormones and salicylic acid content in tomato shoot as affected by some biostimulants at 65 DAS during 2017 season.

Treatments \ Plant hormones	Promoters µg g^{-1} F.Wt.				Inhibitors (Abscisic acid) µg g^{-1} F.Wt.	Salicylic acid µg g^{-1} F.Wt.
	Gibberellins	Auxins	Cytokinins	Total Promoters		
Control	123.67	12.33	340.50	476.50	0.96	2.18
Arginine at 25 mgl^{-1}	126.73	13.10	381.70	521.53	0.38	1.38
Arginine at 50 mgl^{-1}	159.11	15.14	454.28	636.65	0.62	2.91
Tryptophan at 50 mgl^{-1}	103.29	10.98	397.23	511.50	0.67	2.11
Tryptophan at 100 mgl^{-1}	167.23	17.33	43314	609.58	0.57	1.53
Milagrow at 50 mgl^{-1}	154.40	14.62	468.25	637.27	0.55	3.25
Milagrow at 100 mgl^{-1}	118.03	11.60	417.19	546.82	0.84	2.70

Brassinosteroids are steroids which occur in several plant species with biological activities, they are a new plant growth hormones group (Yokota and Takahashi, 1985). Brassinosteroids affect many biological processes, such as stem elongation, leaf bending as well as xylem formation (Yokota, 1997). These activities of development are associated with activity of ATPase (Cerana *et al.*, 1983), 1-aminocyclopropane-1-carboxylic acid synthase (Arteca *et al.*, 1983), alteration of micro tubule orientation (Mayumi and Shibaoka, 1995) and cell walls modification (Zurek *et al.*, 1994). Clouse and Zurek (1991) indicated that exogenously applied brassinolide, the most active brassinosteroid, promoted differentiation of tracheae and cell division. Helal and Gomaa (2007) reported that stigmasterol induced significant a promotive effects on stem and leaf anatomical structure of lupine plant**.** El-Badawy and Abd El-Aal (2013) indicated that tryptophan foliar application improved the most of mango plant leaf anatomical features. Abd El-Aal and Eid (2018) found that foliar application with amino acids at 4 mll^{-1} treatment enhanced leaf and stem anatomical features of soybean plant.

3.3.2 Anatomical characteristics of stem

Data in Table (5) and Figure (2) show that all the levels of treatments significantly increased the anatomical of stem characteristics i.e., thickness of epidermis tissue, collenchyma layers, parenchyma layers, phloem tissue, cambial tissue, xylem tissue, number of xylem rows vascular $cylinder^{-1}$, number of xylem vessels row^{-1}, diameter of widest M. xylem vessel, thickness of parenchymamatous pith and stem diameter. From these results can conclude that, the beast treatment was milagrow at 50 mgl^{-1} which recorded the highest values for thickness of epidermis tissue (17µ), collenchyma layers (251µ), phloem tissue (266µ), cambial tissue (109µ), xylem tissue (841µ) and diameter of widest M. xylem vessel (86µ) respectively, but the concentration at 100 mgl^{-1} recorded the highest values for thickness of parenchymamatous pith (2809µ) and stem diameter (5940µ) comparing with the control. On the other side, the treatment with arginine, recorded low values, especially at 25 mgl^{-1} compared with Milagrow and tryptophan treatments in certain histological features since, thickness of cambial tissue was (57µ), xylem tissue (560µ), number of xylem rows vascular $cylinder^{-1}$ (56), number of xylem vessels row^{-1} (15), thickness of parenchymamatous pith (1962µ) and stem diameter (4551µ) flowed by the treatment with tryptophan, especially at 50 mgl^{-1} for thickness of collenchyma layers (163µ), parenchyma layers (248µ), phloem tissue (181µ) and number of xylem vessels row^{-1} (15) respectively, comparing with the other biostimulant treatments.

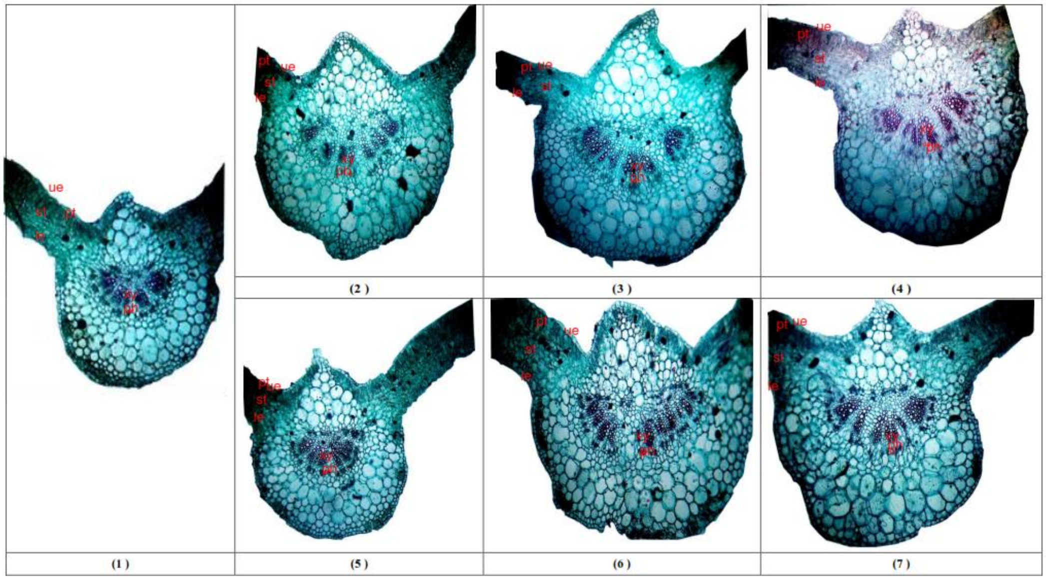

Figure (1): Transverse sections (X = 40) through 4th apical leaf of *Lycopersicon esculentum* Mill plant at 65 DAS as affected by different applied treatments. Where: (1): Control, (2): Arginine at 25 mgl^{-1}, (3): Arginine at 50 mgl^{-1}, (4): Tryptophan at 50 mgl^{-1}, (5): Tryptophan at 100 mgl^{-1}, (6): Milagrow at 50 mgl^{-1}, (7): Milagrow at 100 mgl^{-1}, ue= upper epidermis, le= lower epidermis, pt= palisade tissue, st= spongy tissue, ph= phloem tissue, xy= xylem tissue.

Table (4): Anatomical structure of tomato leaf as affected by some biostimulants at 65 DAS during 2017 season.

Treatments / Histological Characteristics (micron)	Control	Arginine at 25 mgl^{-1}	Arginine at 50 mgl^{-1}	Tryptophan at 50 mgl^{-1}	Tryptophan at 100 mgl^{-1}	Milagrow at 50 mgl^{-1}	Milagrow at 100 mgl^{-1}
Thickness of upper epidermis tissue	21	24	23	19	25	18	22
Thickness of lower epidermis tissue	14	13	15	13	16	16	12
Thickness of palisade tissue	115	112	152	103	174	189	170
Thickness of spongy tissue	162	219	235	187	216	230	181
Thickness of blade	321	376	430	345	463	477	394
Thickness of phloem tissue	206	188	230	192	217	241	225
Thickness of xylem tissue	473	521	610	608	587	592	513
No. of xylem rows	18	19	21	23	20	21	22
Widest of M. xylem vessel	34	42	50	51	44	43	46
Length of midrib vascular bundle	662	690	738	721	727	643	709
Width of midrib vascular bundle	385	423	355	370	488	415	302
Thickness of leaf midrib	1481	1625	1491	1513	1793	1570	1732

Finally, the leaf and stem anatomical characteristics indicate that Milagrow was the beast treatment for enhancement the anatomical structure of tomato leaf and stem i.e., number of rows in the leaf and stem especially at 50 mgl^{-1} as well as increasing the thickness of leaf lamina and midrib especially at 100 mgl^{-1}, subsequently increasing the mineral elements transport and photosynthetic rate. Moreover, the tolerance to adverse conditions especially at high temperature for tomato genotypes during summer season. In general, the stimulatory effects of applied biostimulants on the anatomy features of treated plants could be due to the effect upon cambium activity. Increment of cambium activity could attributed to the increasing of endogenous phytohormones level especially auxins and cytokinins. The above mentioned results especially the conductive tissues increment i.e., xylem and phloem tissues are of great importance because they could be involved in the interpretation about why vigorous growth and high yield fruits were existed with different applied treatments especially with Milagrow at 50 mgl^{-1}. Iwasaki and Shibaoka (1991) mentioned that brassinosteroids producing differentiation of parenchymatous cells into tracheae element. Brassinosteroid is known for acting synergistically with auxin for stimulating cell elongation (Katsumi, 1985, Sasse, 1990). Brassinosteroids enhance sensitivity of tissues to auxin (Mandava, 1988). It is now well known that Brassinosteroids are plant growth regulators which regulate positively plant growth under normal as well as stress conditions (Bajguz and Hayat, 2009, Divi and Krishna, 2009). They regulate the metabolism of growth through the auxin signals for promoting cell division, development of root hairs, differentiation of xylem (Hayat *et al*., 2003). Paschalidis and Roubelakis-Angelakis (2005) indicated that polyamines and their precursor arginine as well as their enzymes biosynthesis involved in stimulation of cell division, expansion and differentiation as well as vascular development in tobacco. Akladious and Abbas (2013) and El-Desouky *et al*. (2011) found that using of amino acids mixture led to an increasing in stem diameter of tomato plant as a result for increasing in the thickness of epidermis and cortex as well as increasing the xylem thickness, especially the vessels number compared to non-treated plant.

3.4 The flowering and yield parameters

3.4.1 Flowering and yield

Data in Table (6) indicate that all the biostimulant treatments significantly increased the flowering and yield parameters i.e., earliness of flower anthesis, number of flowers $plant^{-1}$, pollen grains fertility percentage, early yield number $plant^{-1}$, early yield (g) $plant^{-1}$, total fruits number $plant^{-1}$, fruit setting percentage, abscission percentage, total yield (kg) $plant^{-1}$, fruit fresh weight (g) $fruit^{-1}$ and fruit dry weight (g) $fruit^{-1}$. From these results can conclude that, the beast treatment was milagrow at 50 mgl^{-1} which recorded the highest values for early yield (g) $plant^{-1}$ (731.62), total fruits number $plant^{-1}$ (29.74), fruit setting percentage (37.19), total yield (kg) $plant^{-1}$ (1.94), fruit fresh weight $fruit^{-1}$ (67.38 g) and fruit dry weight $fruit^{-1}$ (3.14 g) flowed by the tryptophan treatment at 100 mgl^{-1} which recorded high values for earliness of flower anthesis (13.70), pollen grains fertility percentage (17.73), early yield number of plant (11.07) in the first season. But in the second seasons all the biostimulant treatments recoded high values comparing with the control. On the other side, the treatment with tryptophan at 50 mgl^{-1} recorded low values for early yield number of plant (9.05), early yield (g) $plant^{-1}$ (605.85), total fruits number $plant^{-1}$ (23.45), fruit setting percentage (32.03), total yield (kg) $plant^{-1}$ (1.58), fruit fresh weight $fruit^{-1}$(65.23 g) and Fruit dry weight $fruit^{-1}$ (2.71 g) comparing with the other biostimulant treatments. In the same trained for the arginine treatment at 25 mgl^{-1} especially in the second season during the same field conditions. Herein, it was clear that the stimulative effects of such treatments on tomato fruits yield was mainly due to their promotional effects on fruits setting and fruits number $plant^{-1}$ rather than fruits weight. This also could be attributed to the pronounced enhancement effects of the same treatments on growth behavior, N, P and K contents, metabolic activity i.e., chlorophyll and carbohydrate contents and the antioxidant bio constituents i.e., carotenoids content. All of them were positively correlated with fruit yield. Milagrow is natural growth promoter. It is growth promoting, increases yield and quality, increases fruit setting percentage, promotes flowering buds formation and resist flowers and fruits dropping.

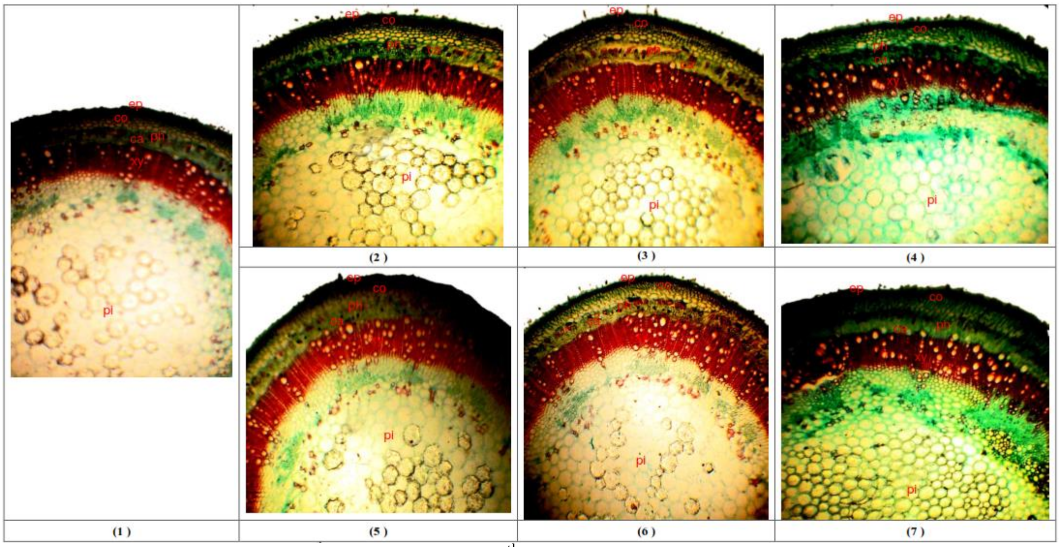

Figure (2): Transverse sections (X = 40) through 4th internode of *Lycopersicon esculentum* Mill plants at 65 DAS as affected by different applied treatments. Where: (1): Control, (2): Arginine at 25 mgl^{-1}, (3): Arginine at 50 mgl^{-1}, (4): Tryptophan at 50 mgl^{-1}, (5): Tryptophan at 100 mgl^{-1}, (6): Milagrow at 50 mgl^{-1}, (7): Milagrow at 100 mgl^{-1}, ep= epidermis, co= cortex, ph= phloem tissue, xy= xylem tissue, ca= cambium pi= pith.

Milagrow found to stimulate flowering buds differentiation, assimilation of carbohydrate and ATP activity, subsequently improving vegetative growth and directed plants to earlier harvest as well as increasing fruit yield and quality characteristics (Gabr *et al*., 2011, Gomes *et al*., 2006, Symons *et al*., 2006). Attia *et al*., (2011) and Seadh *et al.* (2012) recorded that the highest values of safflower capitula number $plant^{-1}$, weight of 100 seed as well as seed yield fed^{-1}

were obtained with milagrow application at rate of 50 ppm. Salazar *et al*., (2016) stated that brassinosteroid treatments increased marketable fruit yield of strawberry plant. The yield increasing in fruit plants may be due to improving the photosynthetic assimilation efficiency of carbon of treated plants. The application of brassinosteroid stimulated activity of photosynthesis expressed with acceleration in CO_2 fixation, increasing protein biosynthesis in wheat and mustard plants and that were related to growth and seed production directly.

Table (5): Anatomical structure of tomato stem as affected by some biostimulants at 65 DAS during 2017 season.

Treatments / Histological Characteristics (micron)	Control	Arginine at 25 mgl^{-1}	Arginine at 50 mgl^{-1}	Tryptophan at 50 mgl^{-1}	Tryptophan at 100 mgl^{-1}	Milagrow at 50 mgl^{-1}	Milagrow at 100 mgl^{-1}
Thickness of epidermis tissue	13	15	16	14	13	17	12
Thickness of collenchyma layers	185	166	217	163	189	251	234
Thickness of parenchyma layers	292	272	280	248	251	274	313
Thickness of phloem tissue	179	215	277	181	265	266	256
Thickness of cambial tissue	54	57	73	83	79	109	68
Thickness of xylem tissue	472	560	794	665	778	841	656
No. of xylem /rows vascular cylinder	74	56	82	77	86	78	65
No. of xylem vessels /row	12	15	18	15	21	19	17
Widest of M. xylem vessel	48	61	72	59	51	86	55
Thickness of parenchymatous pith	2107	1962	2311	2123	2478	2209	2809
Stem diameter	4417	4551	5594	4843	5642	5725	5940

Table (6): Flowering and yield parameters in tomato plants as affected by some biostimulants during 2016 and 2017 seasons.

Characteristics / Treatments	Earliness of flower anthesis (days)	No. of flowers $plant^{-1}$	pollen grains fertility %	Early yield No. $plant^{-1}$	Early yield g $plant^{-1}$	Total fruits No. $plant^{-1}$	Fruit setting (%)	Abscission (%)	Total yield kg $plant^{-1}$	Fruit F.Wt.g $fruit^{-1}$	Fruit D.Wt.g $fruit^{-1}$
Season 2016											
Control	0.00	63.59	10.07	5.39	334.60	21.13	33.23	66.77	1.29	61.05	2.53
Arginine at 25 mgl^{-1}	11.22	76.06	13.85	9.66	642.15	26.59	34.96	65.04	1.76	66.19	3.01
Arginine at 50 mgl^{-1}	10.61	81.75	11.78	10.30	727.23	28.27	34.58	65.42	1.85	65.44	2.95
Tryptophan at 50 mgl^{-1}	8.76	73.21	12.60	9.05	605.85	23.45	32.03	67.97	1.58	65.23	2.71
Tryptophan at 100 mgl^{-1}	13.70	74.23	17.73	11.07	668.13	27.41	36.93	63.07	1.80	65.67	3.07
Milagrow at 50 mgl^{-1}	11.19	79.96	14.39	10.33	731.62	29.74	37.19	62.81	1.94	67.38	3.14
Milagrow at 100 mgl^{-1}	7.42	68.42	15.71	10.72	713.33	26.75	39.10	60.90	1.77	66.17	2.63
L.S.D. 0.05	6.15	4.31	1.06	2.81	91.24	2.67	1.22	1.14	0.28	2.13	0.17
Season 2017											
Control	0.00	69.74	12.32	7.34	378.18	23.09	33.11	66.89	1.17	50.67	2.12
Arginine at 25 mgl^{-1}	9.87	71.46	13.63	11.59	617.04	25.38	35.52	64.48	1.37	53.98	2.66
Arginine at 50 mgl^{-1}	12.26	73.25	15.31	11.84	701.16	31.17	42.55	57.45	1.87	59.99	2.88
Tryptophan at 50 mgl^{-1}	13.52	75.92	16.75	11.09	607.31	27.33	36.00	64.00	1.48	54.15	2.74
Tryptophan at 100 mgl^{-1}	10.17	78.09	16.83	12.35	680.70	30.64	39.24	60.76	1.77	57.77	2.73
Milagrow at 50 mgl^{-1}	15.83	77.59	17.02	13.17	759.56	33.16	42.74	57.26	2.13	64.23	2.91
Milagrow at 100 mgl^{-1}	14.98	72.38	14.77	12.73	722.15	28.87	39.89	60.11	1.62	56.11	2.81
L.S.D. 0.05	4.22	3.18	1.02	3.57	118.56	3.67	1.55	1.08	0.17	3.02	0.44

3.4.2 Fruit chemical compositions

Data in Table (7) showed that all treatments significantly increased the fruit chemical compositions i.e., nitrogen, phosphorus, potassium, crude protein, total carbohydrates, total soluble solid, total acidity and vitamin C mg100^{-1} cm3 juice. From these results could conclude that, the beast treatment was milagrow at 50 mgl^{-1} which recorded the highest values for nitrogen, crude protein, total carbohydrates, total soluble solid and vitamin C. On the contrast, the same treatment was recorded low values for phosphorus, potassium and total acidity comparing with the other treatments during both seasons. Moreover, the results show that, the arginine at 50 mgl^{-1} treatment increased the total acidity (0.44), while it was highly at 25 mgl^{-1}, for phosphorus (0.49) and potassium (3.47) in the first season, but, in the second season for total acidity was highest (0.47) at 25 mgl^{-1}, but, phosphorus (0.45) and potassium (2.72) with arginine at 50 mgl^{-1}, in opposite side, the arginine with 25 mgl^{-1} treatment recorded the low values of the total carbohydrates (60.34), total soluble solid (4.61) g100g^{-1}D.Wt. and vitamin C mg100^{-1} cm3 juice (20.17), especially in the second season compared with other biostimulants.

Table (7): Fruit chemical compositions in tomato fruits as affected by some biostimulants during 2016 and 2017 seasons.

Characteristics / Treatments	g100g^{-1} D.Wt.							Vitamin C 100 mg^{-1} cm^{-3} juice
	N	P	K	Crude protein	Total carbohydrates	Total soluble solids	Total acidity	
				Season 2016				
Control	2.72	0.33	2.61	17.00	57.72	4.09	0.36	19.22
Arginine at 25 mgl^{-1}	2.86	0.49	3.47	17.86	61.73	4.25	0.37	20.46
Arginine at 50 mgl^{-1}	3.16	0.36	2.40	19.75	59.52	4.18	0.44	19.33
Tryptophan at 50 mgl^{-1}	2.91	0.33	2.65	18.19	63.54	5.24	0.38	21.14
Tryptophan at 100 mgl^{-1}	2.95	0.33	2.83	18.44	62.19	5.17	0.40	21.80
Milagrow at 50 mgl^{-1}	3.50	0.32	2.32	21.88	66.17	5.62	0.37	22.41
Milagrow at 100 mgl^{-1}	2.87	0.41	2.18	17.31	64.42	5.02	0.43	21.08
				Season 2017				
Control	2.61	0.43	2.41	16.31	58.61	5.20	0.39	20.11
Arginine at 25 mgl^{-1}	3.46	0.36	2.62	21.63	60.34	4.61	0.47	20.17
Arginine at 50 mgl^{-1}	3.18	0.45	2.72	19.88	63.86	5.25	0.45	21.64
Tryptophan at 50 mgl^{-1}	2.74	0.28	2.25	17.13	62.29	4.16	0.43	20.42
Tryptophan at 100 mgl^{-1}	3.43	0.35	2.36	21.44	62.40	4.62	0.41	20.72
Milagrow at 50 mgl^{-1}	3.71	0.30	2.13	23.19	61.89	5.43	0.41	22.15
Milagrow at mgl^{-1}	2.83	0.32	2.20	17.69	60.81	4.47	0.45	20.81

Finally, from these results we can conclude that, the negative correlation was found among nitrogen, crude protein, total carbohydrates, total soluble solid and vitamin C with phosphorus, potassium and total acidity. The brassinosteroids inducing growth has been related for increasing in RNA and DNA contents, activity of polymerase, synthesis of protein and sugars as well as starch (Kalinich *et al*., 1985, Martin-Tanguy, 1997, Vardhini and Rao, 1998, Vasanth *et* *al*., 2006). Milagrow (Brassinosteroids) has been found for stimulating carbohydrate assimilation and activity of ATP subsequently improving physiological status and directed for increasing fruit yield and quality. It is also evident that they regulate the plant metabolism and yield output.

4. Conclusions

Finally, the beast treatment was milagrow at 50 mgl^{-1} for enhancement the anatomical structure and mineral elements transport, as well as photosynthetic rate, subsequently recorded the highest values for early yield (g) $plant^{-1}$ (731.62), total fruits number $plant^{-1}$ (29.74), fruit setting percentage (37.19), total yield (kg) $plant^{-1}$ (1.94), fruit fresh weight $fruit^{-1}$ (67.38 g) and fruit dry weight $fruit^{-1}$(3.14g), that may be due to enhancing the tomato plant tolerance to adverse conditions especially high temperature during summer season.

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