International Journal of Agricultural and Environmental Research
FREE AND OPEN ACCESS
Available online at www.ijaaer.com
ISSN 2414-8245 (Online)
ISSN 2518-6116 (Print)

LEVERAGING ROLE OF BIOSTIMULANTS ON GROWTH, YIELD AND QUALITY OF TOMATO (*Solanum Lycopersicon*)

MUNAZZA RAFIQUE*[1], SALEEM AKHTAR[1], FAKHAR MUJEEB[1], AND M. ASHFAQ ANJUM[1]

[1]Soil Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan.
**Corresponding author Email: munazzaaari@gmail.com*

Abstract

Nowadays the contests confronted by the agriculture sector are huge. Therefore growing agricultural practices entail more fertilizers for higher crop yield. Presently, to nourish increasing population, extensive requirement for environmental friendly agriculture for the production of healthy and good quality food is in high demand. For a sustainable way of crop production with different organic and botanical fertilizers from natural resources to boost commercially important crop production efforts are under ways. The field experiment was laid out in summer 2017, aimed at evaluating the influence of diverse formulation of natural substances/ biostimulants on crop yield, quality characteristics of fruit and contents of macronutrients in determinate type of tomato (Solanum lycopersicon). Five biostimulant i.e. GB, SM, WK, BM, BS were applied by foliar way along with recommended dose of synthetic fertilizer after transplanting and their performance was recorded during vegetative and reproductive stages. Foliar application of these biostimulants had significant ($p<0.05$) effects on tested parameters of tomato crop over the control. Application of these biostimulant increased plant height (9.0%), No. of branches plant^{-1} (55.5%), fruit yield (17.0%) and quality parameters i.e. total carbohydrates (38.0%), soluble proteins (20.0%), Vitamin C (12.0%), TSS contents (39.0%)over control. Therefore it could be concluded that agricultural biostimulants may be a dynamic approach to boost the yield of tomato and other vegetable crops.

Key words: Agricultural, biostimulants, growth, tomato and yield

INTRODUCTION

Agriculture is an imperative sector of economy world wise, which is grave to the livings of individuals and to the steadiness of the overall global economy. Due to modestly growing of arable acres, agricultural producers will be entailed to become more effectual and fecund to produce ample food for a hungry and growing world. At the same time period, production of crops will remain to be subjected to erratic climates, from flooding to desiccators, extreme heat along with diverse other stresses. To meet future agricultural productivity goals, there are significant progressions across a spectrum of new technologies are required. For recent horticultural production key goal line is an increase in quality of crop. Moreover, along with traditional quality traits, consumers demand towards diet based with increased nutritional value vegetables without the hazards of pesticide remains are now requested. For the last few years, numerous efforts have been laid into emerging new fertilization ways, along with organic farming with lesser standard fertilizer inputs.Nowadays biostimulant products have been introduced in agricultural markets to boost development and growth of the plants with increasing potential of nutrient use efficiency and to improve the productivity, quality, and environmental influence. These are extracts of seaweed, metabolites of bacteria, animal or fungi hydrolysates, which are rich in vitamins, oligosaccharides, humic substances, amino acids, and microelements, (Cavani and Ciavatta 2007). Agricultural biostimulants, has the potential to alter growth of plants positively (Calvo et al. 2014).

Biostimulants are capable to stimulate nutrient uptake and use efficiency by plants, increase plant tolerance to abiotic/biotic stress and improve crop quality when applied in small amounts (De Vasconcelos et al. 2009). Furthermore, biostimulants can enhance the activity of rhizosphere microbes and soil enzymes, the production of hormones and growth regulators in soil and plants, and the photosynthetic process (Calvo et al. 2014).

However, the mechanisms involved in the functional and biological results of plant biostimulants are still unidentified. The diverse nature of the substances and raw materials which is used for production of biostimulant does not let an understanding of the mechanisms involved, or the main component, that are liable for the activity of biostimulants (Nardi et al. 2002). In addition to chemical fertilizer, foliar application of agricultural biostimulant products to plant leaves are not only provide nutrients, but also boost and stimulate various metabolic processes in plant, to increase nutrient use efficiency, or crop yield and quality (Parrado et al.2008). When foliar nutrients are applied, small quantities of chemical fertilizers are required as compared to application of nutrient through soil. Foliar application also reduce leaching or fixation of nutrients. One of the most major benefits of using foliar nourishing is that it is less expensive than various other ways for boosting growth of plant (Dhanasekaran and Bhuvaneswari 2005).

Tomato (*Solanum lycopersicum*) is an important and mainly cultivated vegetable crop. Due to its high nutrient value it has eminent position among vegetable growers. Recently, more efforts have been made to produce organic vegetables. Plant growth regulators and sprinkler nutrient applications are used for increased production of tomato crop. Keeping in view the present study was conducted to aim diverse responses of tomato crop against agricultural biostimulants to meet present agro-challenges.

MATERIALS AND METHODS

Study site and Soil type: A field study was conducted at Agronomy Research Area of Ayub Agricultural Research Institute, Faisalabad, Pakistan. Pre- planting random soil samples of the experimental site were collected (0-30 cm depth), air dried, grounded, thoroughly mixed and kept in the plastic bags for the mechanical and chemical analysis as described by (Page et al. 1982). Sandy clay-loam soil in texture, with pH (soil paste) 7.9 and electrical conductivity (1:5 soil extract) 0.66dsm^{-1}, organic matter 0.69% was used in the experimental field.

Experimental design: Forty five days old tomato plant seedlings with four to five fully matured leaves were planted in an open experimental field. Well plowed soil was fertilized by using standard dose of chemical fertilizer (75-60-60 kg ha^{-1}NPK) with nitrogen as Urea (46%N), Phosphorus source was single super phosphate (18% P), and potassium in the form of potassium sulfate (50%K). Half of nitrogen was applied as basal dose and the remaining was top-dressed on 30th day after planting to all treatments. The experiment was laid out in Randomized Block Design with six treatments and was replicated thrice. Details of treatments are as: 1. Control (tap water), 2. GB (0.1g/L), 3. SM (300-500ml/100L), 4.WK (200-300ml/100L), 5.BM (200-300ml/100L), 6.BS (400ml/100L).

Tomato plants were foliar sprayed at early in the morning with a sprayer (30 l in volume) to run-off, at 25 and 45 d from transplanting after adding tween 20 as a surfactant.

Chemical Composition of Biostimulants Products

Sr No.	Product Name	Composition
1.	GB	Gibberllic acid
2.	SM	Blend of Plant growth regulators
3.	WK	Sea weed extract (Ascophylum nodosum)
4.	BM	Blend of Amino acids
5.	BS	Sea weed extract (Kelp extract)

Data Collection: At 90 days from transplanting, random sample of five plants was taken from each experimental unit to estimate the growth parameters, i.e. (plant height "cm", number of branches per plant).The plant height of tomato crop was measured from the soil level to the tip of the shoot. At harvesting the fruits were manually harvested and determined, single fruit weight, fruit weight per plant and total yield in hectares.

Determination of Photosynthetic pigment concentration: Tomato plant material (0.5 g) was taken (leaf plate without petiole) and homogenized using 100% acetone with pestle and mortar. After filtration and diluting, 25 mL volume, absorbance was measured at 663, and 645 nm with acetone as blank estimation and results were expressed as mg×g^{-1} FW (Yoshida et al. 1971).

Determination of total carbohydrates and soluble proteins: Total carbohydrate in the tomato fruit sample was calculated as described by Sadasivam and Manickam (1996) and soluble protein was estimated by Lowry's method (Lowry et al. 1951).

Determination of Vitamin C percentage (Ascorbic acid) in tomato fruit: The vitamin C contents of fruit (mg 100 g^{-1} juice) were determined using the 2,6-dichloro-indophenol method (Helrich 1990). Tomato fruit mash (25 g) was homogenized in mortar by adding 20 mL of 1% HCl (w/v). Filtrate was dissolved in 100 mL of 1% oxalic acid and 10 mL of aliquots was titrated against 2,6-dichlorophenol-indophenol (Tillman's reagent). The end point of the titration was defined as a pink color that persists through at least 15 s of swirling. Commercial L-ascorbic acid was used as a standard and calculated values were expressed in percentage.

Determination of total soluble solids in the fruits: Tomato juice from the fresh fruit mash was taken for the total soluble solids content determination using digital refractometer. Juice from the sample was squeezed directly onto a refractometer and values were expressed in °Brix units against refractive index.

Determination of total acidity of the fruits: Fruit mash (25 g) was extracted in mortar with dH_2O and homogenate was incubated in water bath at 80°C for 30 min. After filtration, the extract was dissolved in 250 mL of dH_2O. The content of titratable acids was determined by potentiometric titration using 0.1 M sodium hydroxide and phenolphthalein as indicator (Caretto et al. 2008). The values are expressed as percentage.

Determination of ion percentage: For ion percentage; ground dried plant samples were wet digested with $HClO_3/H_2SO_4$, cooled, and brought to the volume of 100 mL using deionized water and kept for ion determination. Total nitrogen was determined by the micro Kjeldahl method. Potassium was determined by a flame photometrically (Kalra, 1998) and phosphorous using ammonium molybdate and ascorbic acid (Coope, 1977).

Determination of IAA concentration: IAA equivalents from the tomato leaf were determined by using Salkowski's reagent as described by Sarwar et al. (1992).

Statistical data analysis:

Data was analyzed using SPSS Statistics 8.1. Analysis of variance (ANOVA) was carried out and significance of differences among the treatments were tested by using least significant difference (LSD).Differences were described significant at Probability level 5%.

RESULTS

Growth parameters: Data presented in table (1) shows that vegetative growth of tomato as plant height, and the number of branches per plant was significantly increased under all biostimulant treatments. It was observed that plant height of tomato crop increased steadily with the development of seedling age. The treatment receiving inorganic fertilizer along with bio-stimulant T2 (Gibberellic acid) recorded the highest plant height (Table 1). Maximum plant height (69.51 cm) was obtained in T_2 as compared to control i.e. 63.01cm. Foliar application of biostimulants significantly increased number of branches per plant when compared to the control. Highest number of branches (9.33) were recorded in T_2 treatment as compared to untreated (6.00).

Floral and yield parameters: Floral characters like days taken to flowering were presented in the Table 2. Minimum (36.33) and maximum (41.00) days taken to flowering were recorded in treatment T_2 and T_1 respectively.

Fruit yield per hectare was significantly higher (41.22t ha^{-1}) with foliar application of GB (gibberellic acid) as compared to control (35.11t ha^{-1}). Fruit length and diameter was also positively increased by application of biostimulants but maximum fruit length was observed in GB and BM (5.54cm) comparing control (5.25cm).In case of fruit diameter , biostimulant treatments are statistically at par but significant response was observed comparing with control as shown in figure,3. Highest fruit diameter was found in BS treatment (4.44%) as compared to control (4.23%).

Quality traits: The biostimulant treatments significantly influenced the quality of tomato fruits. All the treatment were found to be improving the physiochemical parameters *viz.*,total chlorophyll, carbohydrates, soluble proteins, vitamin C and total soluble solids (TSS) contents. T_2 treatment recorded the highest total chlorophyll content (0.56 mg g^{-1}) when compared to control i.e. 0.33 mg g^{-1} (Table 3). Maximum total carbohydrates, soluble proteins contents were obtained in T_3 (2.46, 18.0 mg g^{-1} respectively) as compared to control (1.78, 15.0 mg g^{-1} respectively). Higher vitamin C contents (22.12%) were found in T_4 treatment but it was non-significant to other treatments. Similarly total soluble solids were also high (4.60 %) in treatment where foliar application of WK was carried out as compared to control (3.33%).Fruit acidity was positively effected under all treatments but maximum acidity percentage (0.60%) was observed in treatment where BS was applied comparing with control. Highest value of fresh fruit pulp contents (88.55%) were observed in treatment where BM was sprayed following by BS (88.06%) as described in Figure, 2.

Leaf macro nutrient and IAA contents: Based on the macro nutrient contents of leaves (Table 4), application of biostimulant improve the plant N,P, K. Nitrogen contents of biostimulats treated plants were statistically at par but significant increase was observed as compared to control. Maximum N concentration was obtained in T_3 (5.33%) as compared to control (3.33%). Highest value of P contents were observed in T_4 (0.85%) as compared to untreated plants (0.66%). Significant increase in K contents (4.70%) was found when sprinkler application of biostimulant SM was applied as compared to control (3.07%). In case of IAA contents of tomato leaf, higher value of IAA was observed in BS (3.90 mgg^{-1}) followed by SM (3.70mgg^{-1}) as compared to control .i.e. 2.43mgg^{-1} as presented in figure (1).

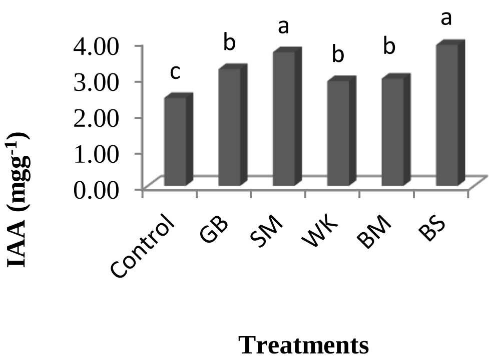

Fig: 1 shows effect of different biostimulants on leaf IAA contents of tomato crop. GB=Gibberllic acid, SM= Blend of Plant growth regulators, WK= Sea weed extract (Ascophylum nodosum), BM= Blend of Amino acids, BS= Sea weed extract (Kelp extract).
***Mean values within a column, followed by different lower letters for main effects are significantly different at P = 0.05 according to LSDtest.*

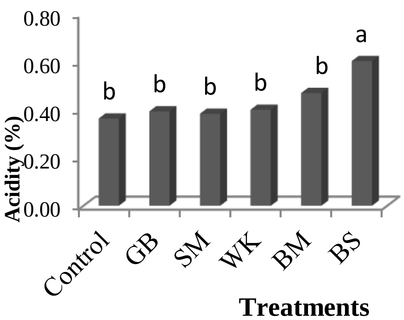

Fig: 2 shows effect of different biostimulants on quality parameters i.e. acidity (%) of tomato fruit respectively. GB=Gibberllic acid, SM= Blend of Plant growth regulators, WK= Sea weed extract (Ascophylum nodosum), BM= Blend of Amino acids, BS= Sea weed extract (Kelp extract).
*** Mean values within a column, followed by different lower letters for main effects are significantly different at P = 0.05 according to LSDtest.*

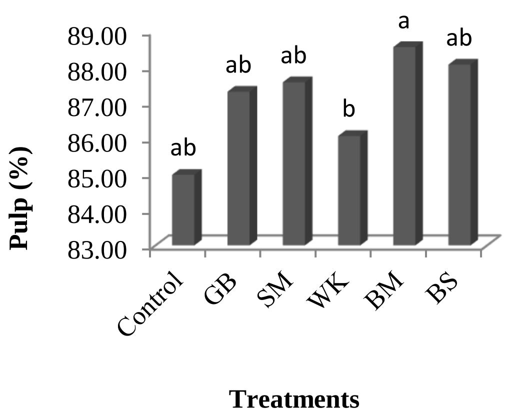

Fig: 3 shows effect of different biostimulants on quality parameters i.e. pulp (%) of tomato fruit respectively. GB=Gibberllic acid, SM= Blend of Plant growth regulators, WK= Sea weed extract (Ascophylum nodosum), BM= Blend of Amino acids, BS= Sea weed extract (Kelp extract).
*** Mean values within a column, followed by different lower letters for main effects are significantly different at P = 0.05 according to LSDtest*

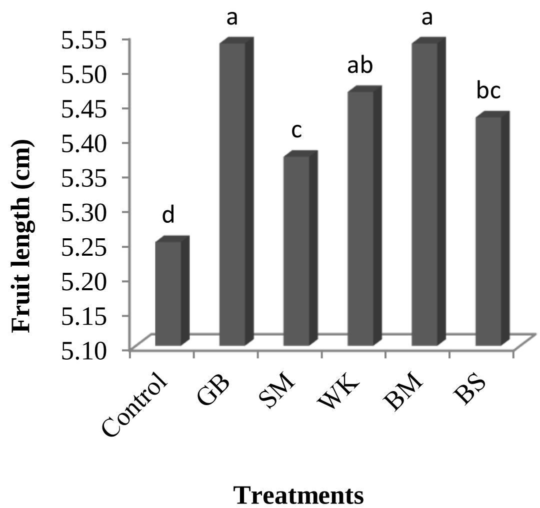

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Fig: 4 shows effect of different biostimulants on growth parameters of tomato fruit. GB=Gibberllic acid, SM= Blend of Plant growth regulators, WK= Sea weed extract (Ascophylum nodosum), BM= Blend of Amino acids, BS= Sea weed extract (Kelp extract).

*** Mean values within a column, followed by different lower letters for main effects are significantly different at P = 0.05 according to LSDtest.*

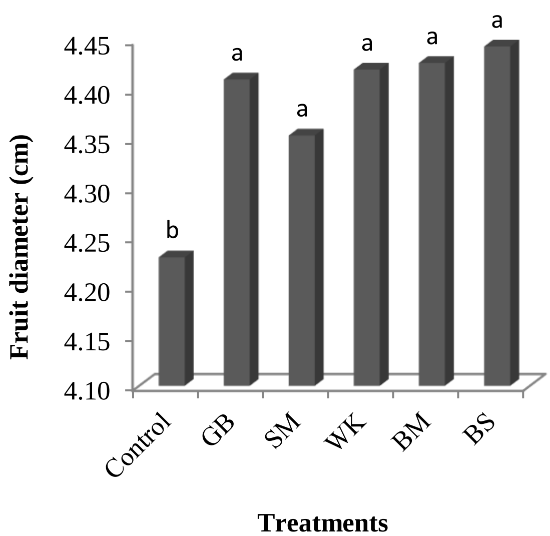

Fig: 5 shows effect of different biostimulants on growth parameters of tomato fruit. GB=Gibberllic acid, SM= Blend of Plant growth regulators, WK= Sea weed extract (Ascophylum nodosum), BM= Blend of Amino acids, BS= Sea weed extract (Kelp extract).

** Mean values within a column, followed by different lower letters for main effects are significantly different at P = 0.05 according to LSDtest.

DISCUSSION

Numerous biostimulants comprise of growth hormones, such as auxins (Pizzeghello et al. 2001; Nardi et al. 2000; Ertani et al. 2012; Jindo et al. 2012), gibberellins, cytokines (Pizzeghello et al. 2013), which are well-

Table.1 Vegetative growth and yield parameters of tomato crop as effected by different bio-stimulants

Characters Treatments	Plant Height (cm)	No of branches plant^{-1}	Days taken for flowering	Single fruit weight (g)	Fruit yield (kg plant^{-1})	Fruit yield (tha^{-1})
Control	63.01±0.61^{c}	6.00±0.45^{c}	41.00±0.58^{a}	35.67±0.88^{c}	1.33±0.04^{e}	35.11±0.59^{c}
GB	68.51±2.05^{a}	9.33±0.56^{a}	36.33±0.67^{d}	41.33±0.33^{a}	1.56±0.02^{a}	41.22±0.62^{a}
SM	69.21±2.16^{a}	7.67±0.78^{abc}	39.33±0.67^{b}	39.67±0.33^{ab}	1.51±0.03^{b}	40.00±0.39^{a}
WK	64.48±0.85^{bc}	7.33±0.88^{bc}	37.00±0.58^{c}	38.67±0.45^{b}	1.49±0.02^{c}	39.55±1.46^{ab}
BM	67.50±1.43^{ab}	8.00±0.54^{ab}	38.66±0.33^{bc}	40.00±0.58^{ab}	1.47±0.01^{c}	39.22±0.40^{ab}
BS	63.78±1.35^{bc}	7.33±1.00^{bc}	40.00±0.58 ^{ab}	41.00±0.79^{a}	1.42±0.02^{d}	37.22±1.18^{bc}
LSD $_{0.05}$	3.7469	1.6828	1.7471	1.8691	0.0209	2.4745

**GB=Gibberllic acid, SM= Blend of Plant growth regulators, WK= Sea weed extract (Ascophylum nodosum), BM= Blend of Amino acids, BS= Sea weed extract (Kelp extract).*
***Mean values within a column, followed by different lower letters for main effects are significantly different at P = 0.05 according to LSD test.*

Table.2 Photosynthetic pigment concentration and some quality attributes of tomato crop effected by different bio-stimulants

Characters Treatments	Total chlorophyll (mg g^{-1})	Carbohydrates (mg g^{-1})	Soluble Proteins (mg g^{-1})	Vitamin C (%)	TSS (%)
Control	0.33±0.02^{e}	1.78±0.01^{d}	15.00±0.58^{b}	19.61±0.90	3.33±0.15^{d}
GB	0.56±0.05^{a}	2.30±0.03^{b}	16.33±0.88^{ab}	19.90±0.71	3.90±0.26^{c}
SM	0.44±0.03^{c}	2.46±0.03^{a}	18.00±0.57^{a}	21.74±0.35	4.37±0.26^{ab}
WK	0.48±0.05^{b}	1.92±0.01^{c}	15.66±0.86^{b}	22.12±1.48	4.60±0.21^{a}
BM	0.46±0.06^{bc}	2.33±0.09^{b}	16.00±0.58^{ab}	21.95±0.38	4.17±0.19^{bc}
BS	0.37±0.01^{d}	1.87±0.01^{c}	15.30±0.76^{b}	20.10±0.53	4.07±0.16^{bc}
LSD $_{0.05}$	0.0215	0.0668	2.0542	NS	0.3215

**GB=Gibberllic acid, SM= Blend of Plant growth regulators, WK= Sea weed extract (Ascophylum nodosum), BM= Blend of Amino acids, BS= Sea weed extract (Kelp extract).*
***Mean values within a column, followed by different lower letters for main effects are significantly different at P = 0.05 according to LSDtest.*

Table.3 Ion percentage of tomato plant effected by different bio-stimulants

Characters Treatments	N (%)	P (%)	K (%)
Control	3.33±0.32^{b}	0.66±0.01^{c}	3.07±0.07^{c}
GB	5.29±0.24^{a}	0.84±0.03^{ab}	4.03±0.03^{b}
SM	5.33±0.33^{a}	0.76±0.08^{b}	4.70±0.15^{a}
WK	5.31±0.27^{a}	0.85±0.04^{a}	3.83±0.07^{b}
BM	4.67±0.25^{a}	0.81±0.05^{ab}	4.17±0.17^{b}
BS	5.00±0.31^{a}	0.77±0.03^{b}	4.63±0.14^{a}
LSD $_{0.05}$	0.9965	0.0758	0.3811

**GB=Gibberllic acid, SM= Blend of Plant growth regulators, WK= Sea weed extract (Ascophylum nodosum), BM= Blend of Amino acids, BS= Sea weed extract (Kelp extract).*
***Mean values within a column, followed by different lower letters for main effects are significantly different at P = 0.05 according to LSDtest.*

known to responsible for the positive effects on plant growth. The mechanism of biostimulants is mostly mysterious and hard to identify, because they originate mainly from multifarious sources containing several bioactive components that together, may pay to particular effects in plants (Ertani et al. 2011a, b). Use of plant growth regulators (PGRs) based biostimulants in horticulture crops to enhance plant growth has much importance. Gibberellins cause plants to grow taller by stimulating cell elongation. When a plant releases gibberellins, its cells initiate a process of elongation (Chen et al. 2002). As plants are mainly composed of single cells that are stacked on top of one another, this elongation of thousands of individual cells results in the overall growth of the plant. Application of GA_3 in tomato crop helps in formation of protein including several enzymes increases level of shoot elongation and increase in photosynthetic capacity, leading to total leaf area and leaf dry weight (Mostafa and Saleh 2006).In this study, it was found exogenous application of plant growth regulator based biostimulant improved growth of tomato plant (Table: 1) which was healthier for nutrient up take compared to the uninoculated control. Rise in plant height may be due to the reason that gibberellic acid enhanced cell division, cell & stem elongation that result in highest vegetative growth (Kanimozhi 2004, Rahman et al. 2004). Bokode et al. 2006 observed that the tomato plants treated with GA_3 50ppm concentration produced maximum height of plant. Hasanuzzaman et al. 2015 revealed that exogenous application of GA_3 @ 125 ppm showed an increase in plant height, number of leaves, dry matter content of stem and root, branches per plant in tomato. Role of biostimulants in other processes, such as stem elongation, seed germination, flowering, and fruit ripening is well known. GA_3 application at 50 ppm was found to be more significant in earliness to 50% flowering. Gibberellin makes cell division, cell elongation and cell enlargement as stated by Chaudhary et al. 2004. Biostimulants also are used widely in horticulture crops to enhance plant growth and ultimately improve yield by increasing number of fruit, fruit set and size. Artificial parthenocarpy induction through application of PGRs allows fertilization-independent fruit development that can diminish yield variability in crops like tomato, pepper and likes (Heuvelink and Korner, 2001). Similarly our results (Table :1) indicated that days taken for flowering and fruit yield significantly effected by foliar application of GB (Gibberellic acid).The increased yield by the foliar application of biostimulants over control is mainly due to availability of nutrients, improvement in cell division, different plant growth hormones production that effects on anabolic activities. Therefore, the nutrients that were earlier utilized by vegetative part were translocated towards reproductive organs, and ultimately increased the production of number of flowers per plant and weight of the fruits (Tiwari et al. 2012). To boost yield and improve fruit quality under unfavorable climatic conditions of high temperature application of GA_3 is important (Gelmesa et al. 2010).It has been described GA_3 was used to stimulate fruit setting and yield in tomatoes (Tonder and Combrink, 2003) Several studies on the effect of biostimulant application in horticultural crop quality parameters are concerned under control conditions .The relationship between chemical composition of fruit and biostimulant application in field-grown tomato is more complicated due to the complex composition of biostimulants and their interaction with plant constituents (Goswami et al. 2013). The present investigation showed that there was a significant increase in chlorophyll concentration (table, 2), biostimulants also elevated the potassium level (table, 3), which might have responsible to an increase in chloroplast per cell (Taiz L and Zeiger E 2003). The role of biostimulants in increasing chlorophyll concentration may be due to containing considerable amounts of macro-and micronutrients, amino acids, vitamins and hormonal like activities (Kurepin et al., 2014) , that possibly increased chlorophyll level leading to higher rates of photosynthesis. These results were confirmed in tomato plant (Zodape et al. 2011). It is documented from the study that foliar application of biostimulants enhanced tomato fruit quality (table, 2). Our results were confirmed by (Calvo et al., 2014). The favorable influences of biostimulants on the chemical characteristics of tomato fruit may be due to its stimulative effect on photosynthesis process and its concentration of some promoter hormones such as cytokinins which are closely involved in cell division, protein, carbohydrates, and chlorophyll formation. It has been found that foliar application of seaweed extract has resulted in an increase in potato tuber quality represented as total acidity, total soluble solids and ascorbic acid content (Arafa et al. 2012).

Our study data also indicated that application of biostimulants significantly increased ion concentration in the plant compared with untreated control (table, 3). It may be due to improving root system, increasing proliferation of root hairs, production of smaller and more ramified lateral roots (Canellas et al. 2002) and to stabilizing membrane permeability, additionally improving nitrogen use efficiency by retarded nitrification processes or inhibited urease activity (Adani et al. 1998). Castaings et al. 2011 indicated that application of sea weeds enhanced nitrogen assimilation.

CONCLUSION

Biostimulants have an advantageous role for field tomato to improve yield, quality by protection of plants against abiotic stresses. Treatment of tomato plants with biostimulants during their vegetative growth stimulate growth processes and influence directly on crop productivity. The present study showed positive and statistically significant effects of biostimulant treatments, however future investigations are needed to clarify the impact of such natural substances on horticultural crops.

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