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Influence of Biostimulants and Plant Growth Regulators on Physiological and Biochemical Traits in Tomato (*Lycopersicon esculentum* Mill.)

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Increasing the yield and quality has been one of the major objectives of scientists working in tomato especially through organic based inputs. Among the various approaches, use of biostimulants and Plant Growth Regulators has been promising in terms of increasing yield and quality in the context of organic farming. Biostimulants are organic compounds composed of peptides, amino acids, polysaccharides, seaweeds, humic acids and phytohormones for immediate uptake and availability within the plant. It also stimulates natural processes to enhance nutrient uptake, tolerance to abiotic stress and crop quality. In order to understand the responses of plants to biostimulants and plant growth regulators in terms of physiological and biochemical changes, the pot culture experiment was conducted in tomato with variety PKM 1. Various components of biostimulants *viz.*, seaweed extract, humic acid, phytohormones were applied as foliar spray at appropriate concentrations in completely randomized block design with four replications. Results revealed that the higher chlorophyll content, soluble protein, NRase activity and shoot length were observed in plants treated with 100 ppm salicylic acid followed by 0.2 per cent humic acid and seaweed- 0.5 per cent *Kappaphycus alvarezii*. These findings suggest that biostimulants and PGRs can be effectively used to influence the physiological and biochemical traits which would results in better growth and yield.

Key words: Tomato, Biostimulants, Seaweeds, Humic acid, Growth regulators

Tomato (*Lycopersicon esculentum* Mill.) is the second most important vegetable next to potato and it is grown widely in tropical regions of the world. Major limitation in tomato productivity is reduced yield, shortened shelf life and poor keeping quality. In order to overcome these limitations, use of compounds like biostimulants and PGRs, has been a promising strategy for enhancing yield, shelf life and quality parameters. Biostimulants have been gaining interest in sustainable agriculture as they activate several physiological processes that enhance nutrient use efficiency, stimulating plant development and allowing the reduction of fertilizers consumption (Kauffman *et al.,* 2007).

Biostimulants are materials, other than fertilizers, that promote plant growth when applied in low quantities (Kunicki *et al.,* 2010). Biostimulants are the extracts obtained from organic raw materials containing bioactive compounds. It also increases root and shoot growth, water uptake and reduces the transplantation shock and helps in soil protection and improves soil health by fostering the development of beneficial soil microorganisms.

Seaweed extracts are nutrient supplements, biostimulants, or biofertilizers, which act as an alternative to chemical fertilizers in agriculture and horticulture to increase plant growth and yield. Seaweed extracts also contain number of phytohormones including auxins, cytokinins, gibberellins, abscissic acid and brassinosteroids. Besides it is also rich in mineral elements like calcium, magnesium, potassium, chlorine, sulphur, phosphorous, iodine, zinc and copper. It is cost effective and maintains eco-friendly environment for sustainable agriculture (Noda *et al.,* 1990). Extracts of brown seaweeds are widely used in horticulture crops for their plant growth-promoting effects and for their ameliorating effect on crop tolerance to abiotic stresses such as salinity, extreme temperatures, nutrient deficiency and drought (Battacharyya *et al.,* 2015).

Humic substances are natural constituents of the soil organic matter, resulting from the decomposition of plant, animal and microbial residues. Humic acid may stimulate plant growth by improving nutrient uptake by exerting hormone-like effects as auxins (Baldotto *et al.,* 2010). Humic acid stimulate shoot elongation and increase leaf nutrient accumulation and chlorophyll biosynthesis (Baldotto and Baldotto, 2013).

Plant growth regulators are the chemical substances which modify the growth and development of the plant by promoting or inhibiting the growth at very low concentration. The plant growth regulators are known to enhance the source-sink relationship

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and stimulate the translocation of photo-assimilates thereby helping better fruit set in many crops. Among the PGRs, auxins and gibberellic acid are considered vital for improving plant growth and productivity and therefore, could be a useful alternative to increase crop production.

Naphthalene acetic acid (NAA) plays an important role in promoting plant growth by enhancing cell division and cell differentiation which may initiate the development of plant organs. Gibberellic acid is one of the growth regulators that have positive effect on plant growth through the effect on cell division and elongation (Batlang *et al.,* 2006). GA_3 increases plant height, shoot and root fresh weight, shoot and root dry weight, root length, root number, relative water content, chlorophyll content, anthocyanin content and increased activity of enzyme carbonic-anhydrase (Al-Whaibi *et al.,* 2010). Salicylic acid (SA) is an endogenous plant phenolic compound which is involved in regulation of plant growth and development and also enhances the abiotic and biotic stress tolerance. Salicylic acid (SA) has a direct physiological effect through the alteration of antioxidant enzyme activities, induction of flowering, increased flower life, delayed senescence and increased cell metabolic rate.

Material and Methods

Pot culture experiment was conducted at the glass house, Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore from October 2017 to March 2018. The experiment was laid out under completely randomized block design with nine treatments and four replications. The seeds of tomato variety PKM 1 were sown in protrays and transplanted on 25 days after sowing. The necessary cultural operations were carried out as per the recommendations.

The crops were foliar applied with the biostimulants and growth hormones as per the treatment schedule *viz.,* T_1- Control, T_2- Seaweed (*Kappaphycus alvarezii*) @ 0.5%, T_3- Seaweed (*Ascophyllum nodosum*) @ 0.2%, T_4- Humic acid @ 0.2%, T_5- SA @ 100 ppm, T_6- GA_3 @ 50 ppm, T_7- NAA @ 40 ppm, T_8- NAA @ 40 ppm + GA_3 @ 50 ppm and T_9- NAA @ 40 ppm + GA_3 @ 50 ppm + SA @ 100 ppm. Foliar applications were given twice at 30 and 50 days after transplanting. The physiological parameters were measured at 40 and 60 days after transplanting. Three plants from each treatment in each replication were selected at random and utilized for recording observations on the following characters *viz.,* chlorophyll content (mg g^{-1}), nitrate reductase activity (µg of NO_2 g^{-1} h^{-1}), soluble protein (mg g^{-1}), shoot length and root length in tomato. Chlorophyll pigment was determined by DMSO method (Hiscox and Israelstam, 1979) at 663 and 645 nm and expressed as mg g^{-1} of fresh weight. Soluble protein content in the leaf was estimated at 660 nm by using Folin Ciocalteau reagent by following the procedure described by (Lowry *et al.*, 1951). Nitrate reductase activity was estimated in fully expanded functional leaves following the method of Nicholas *et al.* (1976). The shoot length of individual plants were expressed from the first cotyledonary node to the tip of the main stem and expressed in centimeters (cm). The root length of each plant was measured from the cotyledonary node to the root tip and expressed in cm. The data were analyzed statistically by the methods outlined by Gomez and Gomez (1984).

Results and Discussion

Experimental results showed that the spraying of biostimulants and plant growth regulators has showed significant improvement in chlorophyll content, soluble protein, nitrate reductase activity, shoot length and root length on both 40 and 60 days after transplanting.

Table 1. Effect of biostimulants and plant growth regulators on biochemical parameters in tomato

Treatment	Chlorophyll content (mg g^{-1})			Soluble protein (mg g^{-1})			Nitrate reductase activity (µg of NO_2 g^{-1} h^{-1})		
	40 DAT	60 DAT	Mean	40 DAT	60 DAT	Mean	40 DAT	60 DAT	Mean
T_1 : Control	1.05	1.16	1.10	6.61	7.12	6.87	127.00	149.35	138.18
T_2 : Seaweed (*Kappaphycus alvarezii*) @ 0.5%	1.37	1.68	1.52	7.40	7.54	7.47	141.00	169.50	155.25
T_3 : Seaweed (*Ascophyllum nodosum*) @ 0.2%	1.34	1.57	1.45	7.12	7.53	7.32	134.00	158.31	146.16
T_4 : Humic acid @ 0.2%	1.37	1.66	1.51	7.58	8.18	7.88	148.00	169.85	158.93
T_5 : Salicylic acid @ 100 ppm	1.48	1.69	1.58	8.46	10.05	9.25	153.98	177.28	165.63
T_6 : Gibberellic acid @ 50 ppm	1.21	1.33	1.27	7.01	7.41	7.21	135.00	157.96	146.48
T_7 : NAA @ 40 ppm	1.23	1.37	1.30	6.98	7.34	7.16	136.00	159.25	147.63
T_8 : NAA @ 40 ppm + Gibberellic acid @ 50 ppm	1.26	1.34	1.31	6.69	7.17	6.93	132.00	148.21	140.11
T_9 : NAA @ 40 ppm + Gibberellic acid @ 50 ppm + Salicylic acid @ 100 ppm	1.27	1.35	1.31	6.99	7.39	7.19	131.00	144.65	137.83
Mean	1.28	1.46	1.37	7.20	7.75	7.48	137.5	159.3	148.46
SEd	0.01	0.01		0.08	0.12		2.20	1.56	
CD (P:0.05)	0.02	0.03		0.16	0.25		4.52	3.21	

The results indicated that tomato plants (PKM 1) sprayed with 100 ppm of salicylic acid has recorded higher chlorophyll content of 1.69 mg g^{-1} followed by seaweed (*Kappaphycus alvarezii*) @ 0.5% 1.68 mg g^{-1} (Table 1). Similarly the foliar application of 100 ppm of salicylic acid resulted in maximum accumulation of chlorophyll content in tomato leaves was reported by (Senaratna *et al.,* 2000). Seaweed extract applied plants maintained longer greenness of leaves, due to the presence of betaine which delays the degradation of chlorophyll in leaf (Crouch *et al.,* 1993). Similarly, the soluble protein content was found to be higher in the treatment, 100 ppm of salicylic acid followed by humic acid @ 0.2 % and seaweed (*Kappaphycus alvarezii* @ 0.5%) (Table 1). The results collaborate with findings of Muthulakshmi *et al.,* (2016), that Salicylic acid @ 150 ppm showed maximum soluble protein content in *Vigna mungo*. Similar findings were reported by Chandra *et al.* (2007) in cowpea.

In the present study, the nitrate reductase activity was higher in 100 ppm of salicylic acid (157.96 µg of NO_2 g^{-1} h^{-1}) followed by humic acid @ 0.2% (169.85 µg of NO_2 g^{-1} h^{-1}) and the least value was recorded in control (149.35 µg of NO_2 g^{-1} h^{-1}) (Table 1). This finding is supported by results of Kalarani *et al.* (2002) who observed higher NR activity in tomato in 100 ppm salicylic acid applied plants. The foliar application of seaweed improves nitrate reductase activity in brinjal (Ramya *et al.,* 2015).

Table 2. Effect of biostimulants and plant growth regulators on morphological parameters in tomato

Treatment	Shoot length (cm)			Root length (cm)		
	40 DAT	60 DAT	Mean	40 DAT	60 DAT	Mean
T_1 : Control	60.78	69.50	65.14	13.45	16.35	14.90
T_2 : Seaweed (Kappaphycus alvarezii) @ 0.5%	63.10	74.25	68.68	21.50	24.35	22.93
T_3 : Seaweed (Ascophyllum nodosum) @ 0.2%	65.25	74.50	69.88	17.30	20.50	18.90
T_4 : Humic acid @ 0.2%	68.25	78.35	73.30	23.70	25.70	24.70
T_5 : Salicylic acid @ 100 ppm	69.75	79.70	74.73	16.75	19.30	18.03
T_6 : Gibberellic acid @ 50 ppm	61.20	72.25	66.73	14.95	16.95	15.95
T_7 : NAA @ 40 ppm	62.85	73.85	68.35	15.12	17.60	16.36
T_8 : NAA @ 40 ppm + Gibberellic acid @ 50 ppm	63.25	73.15	68.20	15.24	18.10	16.67
T_9 : NAA @ 40 ppm + Gibberellic acid @ 50 ppm + Salicylic acid @ 100 ppm	61.12	71.65	66.39	16.50	19.15	17.83
Mean	63.94	74.13	68.99	17.16	19.77	18.47
SEd	0.79	1.04		0.17	0.23	
CD (P:0.05)	1.63	2.14		0.36	0.48	

Increasing trend in shoot length was observed from 40 to 60 DAT (Table 2). Significant increase in shoot length was observed in 100 ppm of salicylic acid followed by humic acid @ 0.2% and seaweed (*Ascophyllum nodosum*) @ 0.2%. Similarly, it was reported by Amin *et al.,* 2008) in wheat plants, which showed increase in shoot length in 100 ppm salicylic acid applied plants. Application of growth promoting substances increases plant height by enhancing the cell division and cell elongation at the shoot apex region which is mainly due to the enhanced photosynthetic efficiency and the mobilization of photosynthates in soybean was reported by (Shukla *et al.,* 1997).

Root length of tomato plant treated with salicylic acid showed an increase in root length significantly till 60 DAT (Fig. 2.). Increase in root length was observed in the treatment T_4 (humic acid @ 0.2%) followed by *Kappaphycus alvarezii* @ 0.5% and *Ascophyllum nodosum* @ 0.2%. Similar results were obtained by Zodape *et al.* (2008) in foliar application of 5% *Kappaphycus alvarezii*. It is due to the presence of growth promoting substances like carbohydrates (Booth, 1965), macro and micro nutrients in seaweed extract and its translocation from shoot to root throughout the growth period.

From the results it could be concluded that, the treatment T_5 - Salicylic acid @ 100 ppm registered highest values in physiological and biochemical parameters *viz.,* chlorophyll content, soluble protein, nitrate reductase activity and shoot length in tomato. The plant growth regulators enhances the source-sink relationship and stimulate the translocation of photo-assimilates resulting in better fruit set in many crops. Therefore, biostimulants and PGRs could be potential compounds to improve plant growth by positively influencing the physiological and biochemical traits.

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