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## Exploitation of Biostimulatants and Vitamins as an Alternative Strategy to Control Early Blight of Tomato Plants

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**Abstract:** Early blight of tomato is a fungal disease, caused by *Alternaria solani* (Ellis and Martin) Jones and Grout, is a major disease of Solanaceae. Therefore, for commercial cultivation in the field controlling early blight is necessary. The use of natural compounds as alternatives or complements to chemical fungicides would be helpful because the crops require continued prolonged harvesting, during which young unsprayed leaf tissue continuously become available for infestation. Several natural compounds were tested on tomato plants grown under field condition and natural infestation to evaluate the potential of biostimulants on improving tomato plant growth, yield and to induce protection from early blight disease. Foliar application of chitosan (Chit), humic acid (HA), seaweed extract (SE) and thiamine (Thi) proved to be effective in reducing early blight disease incidence in tomato plants. All tested biostimulants, in particular, thiamine significantly increased tomato growth parameters (shoot length, no. of branches and leaves per plant, shoot fresh and dry weight and leaf area per plant) and some physiological aspects (nitrogen, potassium, phosphorus and chlorophyll as well as total carbohydrate content) in the shoot. In addition, tomato yield/fed was also increased. Thi at 50 mg L<sup>-1</sup> and SE at 500 mg L<sup>-1</sup> were the most effective in this concern. Thi and SE could be recommended for controlling early blight disease of tomato in the field and improving its growth and yield.

Key words: Tomato, early blight, chitosan, humic acid, thiamine, seaweed extract, growth, yield

### INTRODUCTION

Tomato (Lycopersicon esculentum Mill.) is one of the world's major vegetables with 4.4 million ha under production and 115 million tons produced worldwide in 2004 (FAOSTAT, 2004). It is an excellent source of many nutrients and secondary metabolites that are important for human health: folate, potassium, vitamins C and E, flavonoids, chlorophyll, β-carotene and lycopene and have been linked with reduced risk of prostate and various other forms of cancer, as well as heart diseases (Wilcox et al., 2003). A very common disease in tomato cultivation is early blight caused by Alternaria solani (Ellis and Martin) Jones and Grout. Disease control methods usually involve multiple fungicide applications. The growing popularity of organic production necessitates development and adoption of non-fungicidal approaches for disease control due to the fact that tomato requires continuous prolonged harvesting. There are earlier reports of induction of resistance in horticultural crops through application of elicitors and biostimulants (Farouk et al., 2008; Walz and Simon, 2009). Responses mediated by thiamine (Thi), seaweed extract (SE), humic

acid (HA) and chitosan (Chit) suggested that these plant derived substances have important physiological roles and great potential as elicitors and mediators of resistance signal transduction. They induce unique type of resistance when topically applied and they affect a variety of processes in plants including defense against pathogens (EL-Mohamedy and Ahmed, 2009).

Among the most promising bioactive oligosaccharides is chitosan (Chit) which have attracted tremendous attention because of their unique biological properties, including biocompatibility, non toxicity and biodegradability, their inhibitory effect on the growth of various pathogenic fungi and to stimulate plant growth (Farouk et al., 2008). In the latter studies, a positive effect of Chit was observed on the growth of roots, shoots and leaves of various plants including cucumber plants (Farouk et al., 2008). Moreover, in field studies, application of Chit for inducing resistance against late and early blight diseases of potato and root rot disease of lupine plants was reported by Abd-El-Karem et al. (2004). Trials conducted on tomatoes (Walker et al., 2004) showed that foliar applications of Chit resulted in yield increase of nearly 20% and a significant improvement in

powdery mildew disease control. Recently, Abdel-Mawgoud *et al.* (2010) indicated that Chit application improved plant height, number of leaves, fresh and dry weights of the leaves and yield components. Also, Sheikha and AL-Malki (2011) indicated that Chit works as a positive factor in enhancing shoot and root length, fresh and dry weights of shoots and roots as well as leaves area

Vitamins could be considered as bio-regulators compounds which in little concentration exerted a profound influence upon plant growth, in this concern, Al-Hakim and Alghalibi (2007) indicated that application of Thi increased growth rate, membrane stability and content of photosynthetic pigments. Also, application of Thi counteracts partially or completely the adverse effect of fungal infection (*Fusarium solani* and *Rhizoctonia solani*). Recently, Nahed *et al.* (2010) indicate that on *Thuja orientalis* plants, foliar application of Thi promoted all morphological characters (stem length, stem diameter, root length, fresh and dry weight of root and shoot) and increased total soluble sugar and N, P, K % and protein.

Humic acid "HA" is a suspension which can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against diseases and pests (Scheuerell and Mahaffee, 2006), stimulation plant growth through increased cell division as well as optimized uptake of nutrients and water (EL-Ghamry et al., 2009). Several reports indicated the efficiency of HA in reducing some plant diseases, i.e., chocolate spot and rust diseases of faba bean (EL-Ghamry et al., 2009). Moreover, foliar application of HA increased the content and enhanced the uptake of nutrients in wheat (Seadh et al., 2008).

In Agriculture and horticulture, application of seaweed extract (SE) has proved beneficial for the growth and yield, deeper root development, delay of fruit senescence and improved plant vigour and yield quality and quantity (Zodape et al., 2008, 2011). Furthermore, SE increased plant resistance to pests and diseases, improved plant growth, yield and fruit quality (Jayaraj et al., 2008). There are a few reports available which indicated enhanced plant yield and health in different crops following application, although the mechanisms of action have not been determined. SE contain major and minor nutrients, amino acids, vitamins and also cytokinins, auxine and ABA like growth substances (Crouch et al., 1992) in its extracts, the exact physiological mechanisms are still not known.

The objective of this study was to evaluate the efficacy of four tested biostimulants i.e. Chit, HA, Thi and

SE applied on tomato plants for control of tomato early blight disease under natural infection in the field condition. In addition growth, yield, chlorophyll and total carbohydrates as well as some ions content were determined as a probable response to biostimulants.

#### MATERIALS AND METHODS

Two field experiments were carried out under naturally infection at the Experimental Farm of Plant Pathology Dept., Fac. of Agriculture, Mansoura University, Egypt, during the two successive growing seasons of 2008 and 2009, to investigate the effect of foliar application with biostimulants (chitosan at 500, humic acid at 500, thiamine at 50 and seaweed extract at 500 mg L<sup>-1</sup>) in addition to water as a control treatment on tomato (susceptible cultivar, Super marmand) plant growth and yield as well as some physiological characteristics. Before planting, both physical and chemical analysis for the soil under investigation was undertaken for analysis (Table 1) and Five-week old tomato seedlings were transplanted to the experimental plots on February 28th and March 2nd in the first and second seasons, respectively. All agricultural practices were carried out according to the recommendation of Ministry of Agricultures, Egypt. Complete randomized design with three replicates was allocated. Each replicate consisted of 10 plants spaced at 50 cm apart on both sides of row. The plants from each assigned treatment were sprayed with individual biostimulants twice at 35 and 50 days after transplanting till dripping as well as tape water (check treatment) after adding tween 20 as a wetting agent.

**Disease assessment:** Disease incidence (DI) was measured at 85 days from transplanting.

**Growth parameters:** Two tomato plants were randomly selected from the middle part of each replicate. Shoot length, leaves and branches number per plant, shoot fresh and dry weight and leaf area were determined in the fruiting stage (at 100 days from transplanting).

Chlorophyll determination: Chlorophylls were extracted for 24 h from the 3rd upper leaf at room temperature (at 100 days from transplanting) in methanol after adding traces of sodium carbonate. Chlorophyll concentrations were determined spectrophotometrically (Spekol 11, Uk) according to Lichtenthaler and Wellburn (1983).

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Table 1: Phy	zetochemical	analyete	ot cort	niced in	three ev	meriments

	Physical chara	cteristics								
Properties	Soil texture	Sand (%)	Silt (%)	Clay (%)	CaCO₃ (%)	EC dsm <sup>-1</sup>	Field capacity (%)	Real density	/ (g cm <sup>-3</sup> )	
Values	Clay	19	29	52	3.7	1.43	33	102	24	
	Chemical char	acteristics								
							Available nutrients (ppm)			
Properties	pH soil paste		Organi	ic matter (%)	CEC me	a/100a	N	D	 K	
Values	7.6		1.0		CEC IIIC	ψ100 <u>g</u>	43	14	289	

Ion contents and total carbohydrates: Ion content and total carbohydrates in tomato shoots were determined after 100 days from transplanting. For ion determination, dry shoot samples were digested with HClO<sub>3</sub>/H<sub>2</sub>SO<sub>4</sub> until the solution was clear, cooled and brought to volume at 50 mL using deionized water. Total nitrogen was determined by microkjeldahl method, potassium using flame photometer (Kalra, 1998) and phosphorous using ammonium molybdate and ascorbic acid (Cooper, 1977). On the other hand, total carbohydrates were determined using the anthrone method (Sadasivam and Manickam, 1996).

Fruit yield: Fruit yield was considered as early and calculated as ton /fed.

**Statistical analysis:** Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean±SD for three samples in each group.p-values≤0.05 were considered as significant.

#### RESULTS

**Growth parameters:** Data presented in Table 2 indicated that in both seasons, all growth parameters i.e. shoot length, leaf number and branches number per plant, shoot fresh and dry weights as well as leaf area per plants responded positively and significantly to the application of biostimulants as compared with control plant, except branches number per plant where it is not significantly affected in the second season only.

In both seasons, Thi application gave the greatest values related to growth characters as compared with the other biostimulants and untreated control plants during the two growing seasons. Maximum shoot length (62.53 and 58.20 cm), leaf number per plant (27.6 and 26), branches number per plant (4 and 4 per plant), shoot fresh weight (175.4 and 169.8 g), shoot dry weight (19.70 and 19.14 g) as well as Leaves area per plant (1423 and 1321 cm²) were observed in the treatment of 50 mg L<sup>-1</sup> THI as compared with control plants which gave the lowest plant height (39.53 and 41.56 cm), leaf number per plant

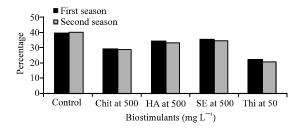


Fig. 1: Disease incidence on tomato leaves as affected by biostimulants under natural infection of early blight after 85 days from transplanting

(14 and 15.33), branches number per plant (2 and 2), shoot fresh weight (120.6 and 120.8 g), shoot dry weight (15.42 and 15.03 g) as well as Leaves area per plant (1071 and 1019 cm<sup>2</sup>), respectively.

Chlorophylls and total carbohydrates contents: Results in Table 3 revealed that spraying of tomato plants with biostimulant, in particular, thiamine, had a significant effect on leaf chlorophyll and total carbohydrate content as compared with untreated control plants. The highest value of chlorophyll and total carbohydrates content in leaves in both seasons was obtained due to application of thiamine followed by seaweed extract. In contrast, the lowest values were obtained from spraying tomato plant with chitosan which sometimes don't differ with control.

**Ion content:** Data presented in Table 4 indicate that application of biostimulants significantly increased ion percentage represented as nitrogen, phosphorous and potassium in tomato shoot. The most effective treatment in this concern was 50 mg L<sup>-1</sup> thiamine which increased nitrogen, phosphorous and potassium percentage from 3.220 and 2.870, 0.399 and 0.393, 1.887 and 1.954% to 5.226 and 4.550, 0.599 and 0.601, 2.806 and 2.895% in the first and second seasons, respectively.

**Disease incidence:** The data illustrated in Fig. 1 during the two growing seasons show that application of biostimulants on tomato plants significantly reduced early blight disease incidence caused by *Alternaria solani* (Ellis and Martin) Jones and Grout as compared to

Table 2: Tomato plant growth as affected by biostimulants under natural infection of early blight after 100 days from transplanting	nt growth as affe	ected by biostimu	lants under nati	ural infection	of early blight	after 100 days fr	om transplanting					
	Shoot length (cm)		Leaf number per plant		Branches number per plant	number	Shoot fresh weight (g)		Shoot dry weight (g)		Leaf area per plant (cm²)	
Treatment ( $\operatorname{mg} L^{-1}$ ) 1st season 2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	39.53±0.272d	39.53±0.272d 41.56±0.600d 14±0.577d	14±0.577d	15.33±0.881d	1d 2±0c	2±0a	120.6±0.692d	120.6±0.692d 120.8±4.46c 15.42±0.195d 15.03±0.057d	15.42±0.195d	$15.03\pm0.057d$		1071±42.1d 1019±32.4c
Chitosanat 500	48.53±0.202c	48.53±0.202c 49.56±0.088c 18±0.577c	18±0.577c	18.33±0.333c	3c 2.33±0.333c	3c 2±0a	149.4±3.40c	149.4±3.40c 145.5±3.62b 17.67±0.121c 17.9±0.311c	17.67±0.121c	17.9±0.311c	$1168\pm6.86c$	1168±6.86c 1156±42.1b
Humic acid at 500	51.26±0.938c	51.26±0.938c 51.2±0.360c 19±0c	19±0c	19.00±0.000c	0c 3±0b	3±0a	$160.3\pm3.58b$	160.3±3.58b 152.5±4.17b 18.28±0.136bc18.36±0.26ba	18.28±0.136bc	18.36±0.26ba		1215±6.86bc 1224±34.4ab
Seaweed Ext. at 500 55.6±1.15b 55.03±0.920b 23.33±0.881b	55.6±1.15b	55.03±0.920b	23.33±0.881b	22.33±1.020b	3.33±0.333b	3b 3±0a	170.4±0.762b	170.4±0.762b 162.5±3.60a 18.74±0.066b 18.67±0.113ab 1250±6.86b 01285±4.15a	18.74±0.066b	18.67±0.113al	b 1250±6.86b	01285±4.15a
Thiamineat 50	62.53±1.70a	62.53±1.70a 58.2±1.00a 27.66±0.881a 26.00±0.577a 4±0a	27.66±0.881a	26.00±0.57	7a 4±0a	4±0a	175.4±1.20a	175.4±1.20a 169.8±4.22a 19.70±0.510a 19.14±0.166a 1423±40.18a 01321±9.31a	19.70±0.510a	$19.14\pm0.166a$	1423±40.18	ı 01321±9.31a
Values are given as Mean±SD of three replicate. Means in columns by different letters are significantly different at p<0.05 by (Duncan's Multiple Range Test).  Table 3: Chlorophyll and total carboly drates content of tomato 3rd upper leaves and shoot as affected by biostimulants under natural infection of early blight after 100 days from transplanting	Mean±SD of thre	ee replicate. Mea 1y drates content	ns in columns b of tomato 3rd up	y different le	tters are signifi nd shoot as affe	cantly different a	at p<0.05 by (Dur. lants under natur	ıcan's Multiple I al infection of e	Range Test). arly blight after	. 100 days from	transplanting	
	Chlorophyll a		Chlorophyll b	hyll b		Chlorophyll a/chlorophyll b	ılorophyll b	Total chlorophyll	phy II	Tota	Total carbohydrates	
Treatment (mg $L^{-1}$ ) 1st season	1st season	2nd season	1st season		2nd season	1st season	2nd season	1st season	2nd season		1st season	2nd season
Control	0.982±0.014b	0.843±0.017d	7d 0.278±0.028c		0.252±0.016c	3.608±0.429a	3.370±0.293a	1.262±0.015d	d 1.096±0.009d		17.26±0.879c	17.47±1.897d
Chitosan at 500	$1.243\pm0.013a$	1.051±0.037c	7c 0.364±0.054c		0.344±0.129bc	3.600±0.643a	3.680±0.975a	$1.607\pm0.050c$	oc 1.396±0.163cd		18.11±1.107c	18.99±1.025c
Humic acid at 500	$1.250\pm0.019a$	1.245±0.018b	3b 0.548±0.040b		0.421±0.097bc	2.306±0.191ab	3.403±0.982a	$1.800\pm0.021b$	b 1.668±0.109bc		19.53±0.462bc	20.36±1.980b
Seaweed Ext. at 500 1.296±0.001a	1.296±0.001a	1.289±0.032ab	2ab 0.621±0.054b		0.545±0.036ab	2.119±0.190b	2.379±0.156a	$1.917\pm0.053b$	.b 1.835±0.054ab		21.69±1.345ab	22.23±0.352a
Thiamine at 50	$1.315\pm0.082a$	1.330±0.001a		0.828±0.079a 0.77	0.771±0.016a	1.633±0.236b	1.726±0.038a	$2.143\pm0.006a$	a 2.101±0.017a		23.86±5.768a	22.85±1.950a
Values are given as Mean±SD of three replicate. Means in columns by different letters are significantly different at p<0.05 by (Duncan's Multiple Range Test)	Mean±SD of thπ	ee replicate. Mea	ns in columns b	y different le	tters are signifi	cantly different a	at p<0.05 by (Dun	ıcan's Multiple l	Range Test)			

Table 4: Nitrogen, phosphorous and potassium percentage of tomato shoot as affected by biostimulants under natural infection of early blight after 100 days from transplanting

	Nitrogen		Phosphorous	Phosphorous		Potassium	
Treatment (mg L <sup>-1</sup> )	1st season	2nd season	1st season	2nd season	1st season	2nd season	
Control	3.220±0.040c	2.870±0.040c	0.399±0.020d	0.393±0.021a	1.887±0.154d	1.954±0.077d	
Chitosan at 500	4.036±0.129b	3.780±0.040b	0.433±0.008c	$0.433\pm0.025b$	2.380±0c	2.402±0.078c	
Humic acid at 500	$4.410\pm0.040b$	4.106±0.269ab	0.459±0.068c	0.448±0.068c	2.537±0.077b	2.492±0.077c	
Seaweed Ext. at 500	4.456±0.269b	4.433±0.199a	0.527±0.044b	0.518±0.043c	2.582±0.134b	2.639±0.139b	
Thiamine at 50	5.226±0.199a	4.550±0.040a	0.599±0.066a	0.601±0.079d	2.806±0.204a	2.895±0.077a	

Values are given as Mean±SD of three replicate. Means in columns by different letters are significantly different at p<0.05 by (Duncan's Multiple Range Test)

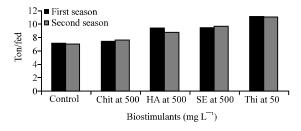


Fig. 2: Tomato yield (kg/fed) as affected by biostimulants under natural infection of early blight after 100 days from transplanting

control treatment. Thiamine at  $50~\text{mg}~\text{L}^{-1}$  followed by Chit at  $500~\text{mg}~\text{L}^{-1}$  showed priority in reducing disease incidence as compared with other biostimulants or control plants in the first and second seasons.

**Tomato yield:** Data illustrated in Fig. 2 clearly show that tomato yield as ton/fed was significantly enhanced with foliar application of biostimulants compared with control. It appeared from the results that thiamine was more effective than other biostimulants application on increased tomato yield in both experimental seasons, where application of thiamine increased tomato yield reached to 11.14 and 10.98 ton/ fed. in the first and second season, respectively as compared with control plant (7.04 and 6.95 ton/fed.).

#### DISCUSSION

Foliar application of biostimulants in both seasons showed, in most cases, a significant increase in tomato growth parameters as investigated in the present study. These increases may be attributed to biostimulant's effect on physiological processes in plant such as ion uptake, cell elongation, cell division, sink/source regulation, enzymatic activation and protein synthesis (Vick and Zimmermann, 1987). Moreover, the positive effect of biostimulants on plant growth may be due to its effect on increasing phosphorous uptake and content as shown in the present investigation. Chitosan (Chit) has specific properties of being environmentally friendly and easily degradable. In agriculture, Chit has been used as

fertilizer and in controlled agrochemical release, to increase plant productivity and to stimulate plant growth under normal and stressed conditions (Farouk et al., 2008). Humic acid (HA) stimulates plant growth by the assimilation of major and minor elements, enzyme activation and/or inhibition, changes in membrane permeability, protein synthesis and finally the activation of biomass production and significantly increased faba bean plant growth (EL-Ghamry et al., 2009). The mechanism by which HA stimulate plant growth are not fully clear, although there are some theories which probably work together. Mechanisms suggested to account for this stimulatory effect of humic substances at the low concentrations reported are numerous, the most convincing of which hypothesizes a "direct" action on the plants, which is hormonal in nature, together with an "indirect action" on the metabolism of soil microorganisms, the dynamics of uptake of soil nutrients especially nitrogen, potassium and phosphorous which are necessary for plant growth and increases in cell permeability (Chen and Aviad, 1990) and soil physical conditions. There is a further possible hypothetical explanation for the hormone-like mode of action of HA in some experiments, there are many researchers extracted plant growth hormones such as IAA, gibberellins and cytokinins from vermicomposts in aqueous solution and demonstrated that these can also have significant effects on plant growth through their involvement in cell respiration, photosynthesis, oxidative phosphorlation, protein synthesis and various enzymatic reactions (Chen and Aviad, 1990). There are several studies proved that application of SE improved significantly plant growth of some plants i.e., tomato plants (Zodape et al., 2011). Vitamins are essential to the metabolism of living organisms. There are many reports on beneficial effects of vitamin application on viability of seedlings, growth and yield of higher plants. Thiamine is connected with the role of thiamine pyrophosphate cocarboxylase, as a co-enzyme in various types of decarboxylation, involving pyruvic and α-keto glutamic acid (Oertli, 1987). Application of vitamins to plants may enhance plant growth and yield by acting as growth regulators under normal conditions (Oertli, 1987) and/or produce hormones and vitamins such

as IAA, thiamine, riboflavin and biotin in addition fixing nitrogen which might stimulates plant growth due to enhancing root development, so it increased plant growth.

The present study indicate that there was a significant increase in chlorophyll content in leaves under different treatments and maximum was recorded under thiamine application. The superiority of biostimulants in chlorophyll content at the early stage may be due to stimulating pigment formation and enhancing the efficacy of photosynthetic apparatus with a better potential for resistance and decrease in photophosphorylation rate usually occurring after infection (Chandra and Bhatt, 1998). Biostimulants were found to increase potassium content (Table 4) which may increase the number of chloroplast per cell, number of cell per leaf and consequently leaf area. This is in harmony with the work carried out by (Farouk et al., 2008) which indicated that application of either thiamine, chitosan or humic acid significantly increased photosynthetic pigments content and activated the synthesis of carotenoids which protect chlorophyll from oxidation and finally increased chlorophyll content as reported in the current study. These results were confirmed in tomato plant (EL-Ghamry et al., 2009; Zodape et al., 2011).

The role of biostimulants on increasing ion content is not fully understood and there are few studies in this field like Farouk et al. (2008) on cucumber and Zodape et al. (2011) on tomato. This increase may be due to enhanced nutrient uptake by improving membrane permeability and/or giving better developed root system. The present investigation clearly shows that, application of any biostimulants increased nitrogen percentage. This increase may be brought about by the amino components in Chit and or higher ability of the plant to absorb N from the soil when Chit was degraded. In addition, application of HA increased nitrogen percentage, this increased was supposed to be due to the better use efficiency of applied N fertilizers in the presence of humic acid coupled with retarded nitrification process enabling the slow availability of applied N (Adani et al., 1998), leads to reduced loss of nitrogen by volatilization. The increase in P uptake as a result of biostimulants application may be due to the prevention of P fixation in the soil and the formation of humophospho complexes, which are easily assimilable by the plants and this explains the excess of P content and uptake by tomato plant in the present study. The highest K uptake was recorded in the treatment application of humic acid. Moreover, there are some reports indicate that application of chitosan increased significantly the content of potassium and phosphorous in plants (Farouk et al., 2008). The present results are in conformity with reported results of nutrient content with

application of SE (Zodape *et al.*, 2011). Concerning the effect of thiamine on ion percentage, the obtained results are in agreement with those reported by Youssef and Talaat (2003) who indicated that foliar application of Thi increased the total nitrogen percentage, total phosphorus % and total potassium % in rosemary plants.

The positive effect of biostimulants on reducing early blight disease incidence may be due to their efficacy as elicitors since localized treatments of plants with biotic or abiotic defense activities can result in localized or systematic responses. Such biostmulants has the ability to inhibit the development of early blight disease in tomato plants. Among natural elicitor compounds, Chit offers a great potential as a biodegradable material that have anti-microbial and elicitation activities. There are numerous reports concerning the protective effects of Chit against pathogen infection in a range of crops (Farouk et al., 2008). The reduction of disease incidence in tomato plants by Chit application may be referred to its propriety as hydrophobic materials, thus creating a low water potential at infected leaf which prevented spore germination, infection and growth when applied before infection as well as, its ability to reduce esterase secretion by pathogens. Moreover, Atia et al. (2005) reported the antifungal activity of Chit against late blight pathogen, which reduced mycelial growth, sporangial production, release of zoospores and germination of cysts of metalaxyl sensitive Phytophthora infestans resistance isolates. Present results indicate that HA acid at concentrations 500 mg L<sup>-1</sup> reduced early blight incidence of tomato plants under field conditions due to enhanced natural resistance against plant diseases and pests (EL-Ghamry et al., 2009). The depression effect of SE on early blight as indicated from the present investigation was confirmed with Zodape et al. (2011) in tomato who indicates that application of SE decreased leaf curl and bacterial wilt occurrence.

The increase in tomato yield may be due to role of biostimulants in stimulation of physiological processes which reflected on improving vegetative growth that followed by active translocation of the photoassimilate from source to sink in tomato plant due to increasing leaf blade thickness and as well as dimension of vascular bundles (unpublished data). Chit has been shown to be a versatile nontoxic material with a dual effect: it controls pathogenic microorganisms and activates several defense responses inducing and/or inhibiting different biochemical activities during the plant-pathogen interaction. To date, there is enough evidence indicating that after Chit application, plants can acquire enhanced tolerance to a wide variety of pathogenic microorganisms, indicating that the use of natural elicitors such as Chit might assist in the goal of sustainable agriculture. It promotes plant growth and enhances crop yield of many species such as cucumber plant (Farouk et al., 2008). Foliar application of humic substances clearly stimulates vegetative growth and yield of tomato. The results are in agreement with those reported for a wide number of plant species (EL-Ghamry et al., 2009). The mechanism of humic acid which stimulate plant growth, yield and quality is not fully clear, although there are some theories which probably work together. a) Application of HA stimulate root growth, increase proliferation of root hairs, production of smaller but more ramified secondary roots and enhancement of root initiation. b) The positive effect of humic on plant growth and productivity, which seem to be mainly due to hormone-like activities of the humic acids through their involvement in cell respiration, photosynthesis, oxidative phosphorylation, protein synthesis and various enzymatic reactions. c)- Humic substances associated with enzymes, can lead to the enhancement of activity of many enzymes (Phosphorilase, phosphatase and cytochrome oxidase), to the inhibition of others (IAA oxidase, fitase and peroxidase) and to the synthesis of some such invertase (Vaughan and Ord, 1985). Enhanced uptake of mineral nutrients content (Farouk et al., 2008). As indicated from the present investigation the treatment of tomato plant with SE increased plant growth and yield. Similar results in tomato with application of SE are reported (Zodape et al., 2011). This suggests a two-fold action of SE: (i) to stimulate root growth at the expense of shoot growth and (ii) to increase the overall photosynthetic accumulation efficiency of the plant. Increased root growth following seaweed treatment is well documented (Crouch and van Staden, 1991). Also, increased yield may be due to increased fruit set and latter fruit weight through better plant canopy establishment, better inception of light and through significant reduction in inter plant competition for solar energy and soil nutrients. Thiamine could serve as coenzyme in decarboxylation of a-keto acids, such as pyruvic acid and keto-glutamic acid which has its importance in the metabolism of carbohydrates and fats (Youssef and Talaat, 2003) reported that pronounced increases in vegetative growth and chemical constituents of rosemary plants by foliar application of thiamine. There is more promise for use of non-chemical approaches in crop production in the light of recent shift towards organic farming and growing public concern to minimize the use of chemical fungicides. Since greenhouse tomato is a commercially important crop, there is more scope for use of alternative strategies for crop protection and they are ultimately economically viable. The advantage of

using biostimulants, in particular, Thi is that it conditions the plant and triggers and contributes optimum level of resistance to multiple pathogens apart from promoting plant growth. This particular potential widens the scope of biostimulants for use in other greenhouse crops also. However, extensive studies need to be conducted under large-scale conditions and locations to evaluate their effectiveness for disease control and improving plant growth and yield in other crops.

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