Induction of Systemic Resistance in Tomato against *Alternaria solani* by Biostimulants and Vitamins

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ABSTRACT

Two field experiments were carried out during the two summer growing seasons of 2008 and 2009, at the Experimental Farm of Plant Pathology Dept., Fac. of Agriculture, Mansoura University, Egypt, to investigate the effect of foliar application of chitosan, humic acid, seaweed extract and thiamine on inducing resistance against *Alternaria solani* to reduce its incidence in tomato plant.

Results indicated that the foliar application of biostimulants decreased hydrogen peroxide accumulation and suppressed the increase in electrolyte leakage percentage.

Early blight disease tolerance in tomato plants was significantly enhanced by increasing catalase and peroxidase activities as well increased phenols, proline and total soluble sugars contents as a direct result to biostimulants foliar application.

Thiamine at 100 mg/l and seaweed extract at 1000 mg/l were the most effective in this concern. These results confirm our primary laboratory findings which indicated incorporating different biostimulants to PDA caused significant reduction in mycelial growth of *Alternaria solani* fungus in comparison with the check treatment.

It could be recommended that foliar application of biostimulants especially thiamine and seaweed extract of tomato plants will induce resistance to early blight and could be used widely because these agents are cheap, available, easy to use and environmental friendly.

Keywords: tomato, humic, seaweed, thiamine, chitosan, systemic acquired resistance, early blight.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the world's most and popular important vegetable crops, comprising Egypt, not only because of its economic importance, but also for the nutritional value of its fruits, mainly due to the fact that they are an excellent sources of natural colors and antioxidants compounds (carotene, lycopene, ascorbic and phenolic compounds). The intake of these compounds in food is an important health protecting factor. They have been recognized as being beneficial for prevention of widespread human diseases including cancer and cardiovascular diseases, when taken in adequate amounts (Fraser and Bramley, 2004). Early blight caused by *Alternaria solani* (Ellis, and Martin) Jones and Grout, is one of the most threatening fungal diseases (Abada *et al.*, 2008b), which causes great reduction in the quantity and quality of fruit during the season. In addition, the disease is favoured by warm temperature and extended periods of leaf wetness from dew, rainfall and crowded plantation. The plants are more susceptible to infection by the disease during fruiting period (Cerkauskas, 2005 and Momel and Pemezny, 2006). It is well known that tomato fruits are mostly consumed freshly, thereby spraying fungicides just before harvesting resulted in high fungicide residue in the fruits, which causes great hazard to human health.

Therefore, this work was mainly planned to evaluate the efficiency of the alternation of spraying some fungicides and antioxidants on reducing the natural infection of tomato early blight.

Plant responds to pathogen attack by activating a wide variety of protective mechanisms designed to prevent pathogen replication and spreading (Malolepsza and Rozalska, 2005). The defense mechanisms including the fast production of reactive oxygen species (ROS) (Asada, 2006) and accumulation of antimicrobial secondary metabolites known as phytoalexins (Agrios, 2005). The generation of ROS such as superoxide anion and hydrogen peroxide are a common event associated with normal plant biochemical processes (Zhou *et al.*, 2004), and also causes oxidative damage through action such as lipid peroxidation with membrane destruction, protein inactivation and DNA damage (Gao *et al.*, 2008). H_2O_2 generation have direct antimicrobial activity inhibiting germination of spores of many pathogens and participation in the formation of phenoxyl-radicals during phenol-polymerization within the plant cell wall (Lamb and Dixon, 1997). Estimation of malondialdehyde (MDA) amount, which is a secondary end product of polyunsaturated fatty acid oxidation, is widely used to measure the extent of lipid peroxidation as indicator of oxidative stress and membrane damage (Lin and Kao, 2000). It was reported that accumulation of MDA and H_2O_2 was

caused by abiotic stress factors such as salinity and drought (Radic´ *et al.*, 2006; Farouk; 2009), heavy metals (Farouk, 2011) and some biotic stress factors such as virus infection (El-Moshaty *et al.*, 1993). (Moreover, Radwan *et al.*, 2006) reported the formation of elevated amounts of MDA and H_2O_2 in *Cucurbita pepo* leaves indicating lipid peroxidation and oxidative stress in response to ZYMV infection. On the other hand, development of an antioxidants defense system in plants protects them against oxidative stress damage by either the partial suppression of ROS production, or the scavenging of ROS which has already been reduced (Cavalcanti *et al.*, 2007). Thus various antioxidants enzymes such as peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) participate in ROS metabolism during the pathogen attack. POD may be some of the elements of the defense systems that are stimulated in plants in response to pathogen infection (Morkunas and Gmerek, 2007). Peroxidases and catalases counteracted the increased production of hydrogen peroxide (Hameed *et al.*, 2008) and were central in plant antioxidant defense response (Hameed *et al.*, 2009). Such associations in disease resistance and biochemical parameters have been suggested to be used as reliable selection tools to identify potential breeding material and to plan effective breeding strategies (Tyagi *et al.*, 2008).

Recent efforts have focused on utilizeing a scheme of inducible plant defenses in order to provide protection against *A. solani* in tomato. Pre-treatment of plants with biotic or abiotic inducers can enhance resistance to subsequent attack not only at the site of treatment, but also in tissues distant from the initial infection sites. Typically, this inducible resistance system known as systemic acquired resistance (SAR) is effective against diverse pathogens (Ryals *et al.*, 1996 and Abdalhadi, 2011). Several natural chemical agents have been described as activators of disease- related processes when applied to plants (Abdulgader, 2011 and El-Samra *et al.*, 2011). Responses mediated by thiamine (THI), seaweed extract (SE), humic acid (HA) and chitosan (CHI) 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 typically applied and they affect a variety of processes in plants including defense against pathogens Jayaraj *et al.*, (2008) and El-Mohamedy and Ahmed (2009).

Among the most promising bioactive oligosaccharides is chitosan (CHI), 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 (Prapagdee *et al.*, 2007 and Farouk *et al.*, 2008) and their ability to be potent elicitors of plant defense reactions (Sharathchandra *et al.*, 2004). Recently, the antioxidant activity of chitosan has also attracted attention (Park *et al.*, 2004). Chitosan can scavenge OH and O_2 - radicals and has been shown to have DNA-protective properties (Harish Prashanth *et al.*, 2007). In addition, treatment of *Hydrilla verticillata* with chitosan has been shown to increase the activity of superoxide dismutase (SOD) and to decrease malonaldehyde (MDA) concentrations (Xu *et al.*, 2007). The scavenging mechanism of chitosan may be related its structure, which features large numbers of hydroxyl and amino groups available to react with ROS (Sun *et al.*, 2004). CHT treatment induced a significant increase in the activities of polyphenol oxidase (PPO) and peroxidase (POD), and enhanced the content of phenolic compounds in tomato fruits, thus providing protection against both gray and blue mould diseases (Liu *et al.*, 2007). Field application of chitosan for inducing resistance against late and early blight diseases of potato and root rot disease of bean and lupin plants was reported by Abd El-Kareem *et al.*, (2002 and 2004a).

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, 2004 and 2006), stimulation plant growth through increased cell division as well as optimized uptake of nutrients and water (Chen *et al.*, 2004). In this respect, El-Ghamry *et al.*, (2009) reported that application of 2000 ppm HA decreased significantly the damage of chocolate spot and rust diseases of faba bean.

Seaweed extract (SE), as foliar nutrient sprays, have been used in horticulture for several decades (Blunden, 1991). Studies conducted with SE spraying under controlled experiments resulted in increased leaf size in spinach, improved root growth in tomato (Verkleij, 1992) and high earlier yield and large fruits size in tomato (Farouk *et al.*, 2012). Furthermore, SE increases plant resistance to pests and diseases, improves plant growth, yield and fruit quality. Finally, THI as an antioxidant (Frederikse *et al.*, 1999), induced disease resistance in plants (Norris and April, 1991).

During the last 20 years this substance has drawn the attention of researchers because of its ability to induce systemic acquired resistance (SAR) in plants.

Therefore, the present study was an attempt to carry out investigations on the effect of biostimulants on tomato plants under natural early blight infestation, with the aim: 1) to characterize the variation in the antioxidant ability of different biostimulants under early blight infestation; 2) to investigate the possible mechanisms responsible for early blight disease tolerance in plants treated with

biostimulants and 3) elucidate the possible mechanisms that might be involved in the biostimulants-promoted antioxidant responses to early blight disease infestation.

MATERIALS AND METHODS

1-Testing the effect of Different biostimulants (HA, SE, THI, CHI) on growth and dry weight of *Alternaria solani*:

1.1. Testing on Linear Growth of *Alternaria solani*:

Different biostimulants of humic acid, seaweed and chitosan were tested to study their inhibitory effect on linear growth of *A. solani* at the concentration 500 mg/l,and thiamine at 50 mg/l . They were added individually to conical flasks containing sterilized PDA medium then mixed gently and dispensed in sterilized Petri plates (9- cm – diameter). Plates were individually inoculated at the center with equal disks (9-mm- diameter) of 10-days old culture of *A. solani*. Five plates were used as replicates for each particular treatment. Inoculated plates were incubated at 25 ± 2 °C.The average linear growth of the fungus was calculated after 10 days.

1.2. Testing the inhitory effect on dry weight of *Alternaria solani*:

Different biostimulants (HA, SE, THI, CHI) were incorporated into 50 ml PD media. A 5 mm diameter agar disc containing fungal mycelium of *Alternaria solani* was transferred to the test medium. Then flasks were incubated at 25°C in a growth chamber. Dry weight was measured after 10 days, when the control flask reached full growth and the average dry weight was measured. Each treatment was represented by 5 flaskes as replicates.

Two field experiments were carried out under natural 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. The experiment was carried out to investigate the effect of foliar application with biostimulants (chitosan, humic acid, seaweed extract and thiamine at the rate of (500,500,500 and 50 mg/l) in addition to water as a control treatment, on inducing tomato (susceptible cultivar, Super Marmand) resistance to early blight. Before planting, both physical and chemical analysis for the soil under investigation was undertaken for analysis and the corresponding data are presented in Table 1.

Five week- old tomato seedlings were transplanted to the experimental plots on February 28th and March 2nd in the first and second season, respectively. All agricultural practices were carried out according to the recommendation of Ministry of Agriculture, 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, 35 and 50 days after transplanting till dripping as well as tap water (check treatment) after adding tween 20 as a wetting agent. All physiological characters were measured after 85 days from transplanting.

2. Oxidative damage and stress injury

Hydrogen peroxide (H_2O_2), lipid peroxidation (LPO), malondialdehyde (MDA) and membrane permeability (EC%) in shoot samples were measured to assess the oxidative damage and stress injuries. Lipid peroxidation was estimated content "MDA" produced by thiobarbituric acid reaction as described by Shao *et al.*, (2005) and expressed as MDA content in µmoles/g of fresh weight from the extinction coefficient of 155 mM^{-1} cm^{-1}. A hydrogen peroxide level was estimated by forming a titanium-hydro peroxide complex via methods outlined by (Rao *et al.*, 1997). Electrolyte leakage percentage measurement (ELP) was used to assess membrane permeability according to Goncalves *et al.*, (2007), using an electrical conductivity meter (Hanna, UK). Flag leaf samples were placed in vials containing distilled water and incubated at room temperature for 24 h. Electrical conductivity of the resulting solution (EC_1) was recorded after incubation. Samples were then placed in a boiling water bath for 30 min, cooled to room temperature, and then the second reading (EC_2) recorded. The ELP was calculated as EC_1/EC_2 and expressed as percentage.

3. Assay for ROS scavenging (enzymatic and non-enzymatic)

Catalase (CAT) (EC 1.11.1.6) activity was assayed by measuring the rate of disappearance of H_2O_2 using the method of Velikova *et al.*, (2000). Peroxidase (EC 1.11.1.7) activity was assayed by the method of Reuveni and Reuveni (1995). Ascorbic acid was extracted from plant material and titrated using 2.6- dichlorophenol indophenol as described by (Sadasivam and Manickam, 1996). Total phenolic compounds were quantified by using the method of (Singleton and Rossi, 1965), using Folin-Ciocalteau reagent, that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex. The values were expressed as mg gallic acid/g fw. Proline content was assessed using the procedure decribed in Bates *et al.*, (1973). Briefly, 0.05 g of frozen leaf tissue was homogenized in 5% sulphosalicylic acid. After extraction, homogenates were centrifuged to pellet cell debris at 40°C and 1 ml aliquot of the supernatant was combined with an equal volume of glacial acetic acid and ninhydrine reagent. This mixture was boiled in water bath for 1 h. and then cooled in an ice bath. The solution was partitioned against 2 ml toluene and absorbance at 520 nm measured in this organic layer on a spectrophotometer.

Table 1: Physiochemical analysis of soil used in three experiments

Physical characteristics								
Properties	Soil texture	Sand (%)	Silt (%)	Clay (%)	CaCO3 (%)	EC dsm-1	Field capacity (%)	Real density (g/cm3)
Value	Clay	19	29	52	3.7	1.43	33	1024

Chemical characteristics						
Properties	pH soil paste	Organic matter (%)	CEC meq/100g	Available nutrients (ppm)		
				N	P	K
Value	7.6	1.65		43	14	289

Total soluble sugars extracted by ethanol and then determined by phenol-sulphuric acid method as described by (Sadasivam and Manickam, 1996).

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean LSD for three samples in each group. P values at 0.05 were considered as significant.

RESULTS AND DISCUSSION

1. Effect of Biostimulants on Fungal Growth:

Data in Table (1) showed that all biostimulants applications caused significant reduction in boty weight and linear growth of *Alternaria solani* in comparison with check treatment. In this concern, the most effective treatment was 50 mg/l thiamine which decreased mycelium dry weight and linear growth from 0.950 g and 8.500 cm to 0.500 g and 5.166 cm.

There is strong evidence that mycelial growth can be inhibited or retarded when the growth media of fungi are amended with chitosan (El-Ghaouth *et al.*, 1992c). Other studies reported a complete growth inhibition of fungi such as *F. oxysporum*, *Rhizopus stolonifer*, *Penicillium digitatum* and *Colletotrickum gleosporioides* at concentrations of 3% (Bautista-Banos *et al.*, 2003 and 2004b). These results were in agreement with (Bautista *et al.*, 2003) and Rabea *et al.*, (2003), who reported that chitosan induced multiple biological reactions including induction of phytoalin synthesis of β-1,3 gluconase and chitinase. The mode of action of chitosan as fungicide might be explained by its interaction with the fungal DNA and/or RNA as stated by Hadwiger and Loschke, (1981). Additionally, Leuba and Stossel, (1986) indicated that the antifungal activity of chitosan is related to its ability to interfere with the function of plasma membrane of fungal cells.

2. Effect of biostimulants on oxidative damage:

The presented data in Table (2) clearly showed that, application of biostimulants significantly decreased hydrogen peroxide accumulation. The most effective treatment in this concern was humic acid at 500 mg/l which reduced hydrogen peroxide from 20.636 and 21.367 to 13.667 and 13.989 in the first and second season, respectively. The same table indicated that biostimulants treatments suppressed the increase in ELP.

The production of ROS is one of the earlist cellular responses following successful pathogen recognition. As shown in Table (2) natural inoculation by *Alternaria solani* (Ellis and Martin) Jones and Grout significantly increased level of hydrogen peroxide production in tomato leaves, where ROS was considered as the first defence line against pathogen and may act as direct antimicrobial against pathogen attack (Shetty *et al.*, 2007). Therefore, high production of H_2O_2 in tomato leaves is an important element of disease resistance mechanism which are involved directly or indirectly in restricting pathogen growth and giving the time for plant to moblize further defence reactions. Application of compounds markedly decreased level of hydrogen peroxide in tomato leaves as compared with natural infection. Induction or suppression of ROS generation in leaves of these treatments could be related to the activity of antioxidant enzymes (Table 3), which decreased level of H_2O_2. Thus, our results suggested that the high production of ROS and the capacity of plants to control its concentrations might contribute to increase resistance against *Alternaria solani* infection and giving the time for plant to moblize further defence reactions. ROS have been involved in plant defence

Table 1: Effect of biostimulan on dry weight and linear growth of *Alternaria solani*

Treatment (mg/l)	Dry weight (g)	Linear growth (cm)
Control	0.950 ± 0.850 a	8.500 ± 1.000 a
Chitosan, 500	0.666 ± 0.115 b	6.333 ± 0.577 b
Humic acid, 500	0.733 ± 0.230 b	6.333 ± 0.577 b
Seaweed Ext., 500	0.833 ± 0.115 ab	6.500 ± 1.000 b
Thiamine, 50	0.500 ± 0.200 c	5.166 ± 0.577 c

Values are given as mean± SD of three replicats. Means in columns by different letters are significantly different at $P < 0.05$ by Duncan's Multiple Range Test.

Table 2: Hydrogen peroxide (μM/g fw), Lipid peroxidation (mmole/g fw) and membrane permeability (%) of tomato shoot as affected by biostimulants under natural infection of early blight after 100 days from transplanting.

Treatment (mg/l)	Compound					
	Hydrogen peroxide		Lipid peroxidation		Membrane permeability	
	season					
	1st	2nd	1st	2nd	1st	2nd
Control	20.636 ± 1.471a	21.367 ± 2.551a	14.593 ± 2.181a	15.234 ± 0.484a	86.709 ± 1.241a	87.092 ± 0.487a
Chitosan, 500	18.056 ± 1.793b	18.200 ± 2.797b	7.179 ± 0.587b	7.307 ± 1.000b	80.030 ± 2.688b	80.865 ± 2.336b
Humic acid, 500	13.667 ± 4.897c	13.989 ± 4.738c	6.153 ± 0.257b	6.174 ± 0.196d	75.853 ± 4.223c	76.856 ± 2.507c
Seaweed Ext., 500	16.534 ± 1.753b	16.782 ± 0.855b	6.452 ± 0.195b	6.516 ± 0.195cd	66.832 ± 5.419d	68.890 ± 7.247d
Thiamine, 50	17.756 ± 0.466b	17.834 ± 0.192b	6.730 ± 0.128b	6.815 ± 0.323c	84.304 ± 2.350a	84.788 ± 1.771a

Values are given as mean± SD of three replicats. Means in columns by different letters are significantly different at $P < 0.05$ by Duncan's Multiple Range Test.

responses in several ways:a) reinforcing plant cell wall through cross-linking reactions of lignin and protein, b) acting as toxic agent either on plant cells, with development of HR and SAR, or against the pathogen through killing it or stopping its growth, and c) participating as a second messengers in signaling routes leading to the activation of plant defence related genes (Shinogi *et al.*, 2003).

When plants are subjected to pathogen infection, the equilibrium between productions and scavenging of ROS is broken, resulting in oxidative damage of protein, DNA and lipids. MDA is the marker for lipid peroxidation released from cellular membranes and is formed by the reaction of ROS with lipid molecules (Shinogi *et al.*, 2003). Thus lipid peroxidation in tomato plants has been proved to be induced by pathogens, and the subsequent products have been shown to pass antimicrobial properties (Croft *et al.*, 1993) and signalling function (Melan *et al.*, 1993). In treated plants, MDA levels markedly decreased as compared with infested plants. However, reduction in lipid peroxidation in infested treated plants might be related to the high activity of antioxidants enzymes (Table 3), which preventing the accumulation of free radical, and consequently membrane damage.

The antioxidant properties of chitosan are primarily attributed to its abundant active hydroxyl and amino groups, which react with ROS to form stable and elatively nontoxic macromolecules radicals (Sun *et al.*, 2004 and Sun *et al.*, 2008). Previous studies have applied the antioxidant benefits of chitosan mainly to the fields of biomedicine, food and environmental protection. In the current study, we demonstrated that the antioxidant properties of chitosan can also resistance to oxidative stress in plant subjected to natural infection with *A. solani*. We show that chitosan can mitigate the effects of infection by reducing the extent of lipid peroxidation and by promoting the activities of antioxidant enzymes (CAT and POX).In this concern Yang *et al.*, (2009) reported that application of chitosan suppressed the increase in MDA concentration in plant tissue. In recent years, a growing attention has been directed towards the antioxidant activity of chitosan (Sun *et al.*, 2006 and Sun *et al.*, 2008). Water-soluble chitosan was shown to be an excellent scavenger of hydroxyl radicals, H_2O_2 and anion superoxide (Sun *et al.*, 2008).

3.1. Effect of biostimulants on antioxidant enzymes:

Plants protect themselves against oxidative stress by the synthesis of various antioxidant enzymes. It was observed that antioxidant enzymes activity was higher in biostimulants-sprayed plants compared with control. Exogenous thiamine is more capable than other exogenous biostimulants in enhancing the activities of catalase and peroxidase under natural infection. Thiamine at 50 mg/l is more effective than other biostimulants in mitigating the detrimental effects of pathogen infection by upregulation of antioxidant enzyme activities. The highest increase was observed in 50 mg/l thiamine.

Under pathogen infection, catalase and peroxidase (Table 3) activities increased significantly in the presence of biostimulants, in particular, 50 mg/l thiamine. These results are consistent with the results of Zaky *et al.*, (2006). The present investigation suggests that biostimulants are able to effectively detoxify H_2O_2 by enhancing the activities of catalase and peroxidase under fungal infection. Yang *et al.*, (2009) proved that application of chitosan increased antioxidant enzymes activities. Exogenous application of chemical biostimulants increased the

Table 3: Catalase and peroxidase activities (unit/g fw) in tomto shoots as affected by biostimulants under natural infection of early blight after 100 days from transplanting.

Treatment (mg/l)	Compound			
	Catalase		Peroxidase	
	seasons			
	1st	2nd	1st	2nd
Control	38.402±4.546d	37.432±3.954d	13.796 ± 6.293c	12.830±3.919d
Chitosan, 500	43.581 ±2.918c	42.718 ±4.041c	18.609±2.315b	18.024 ±1.478c
Humic acid, 500	46.385±0.988b	46.17 ±1.628b	22.708 ±4.341a	22.67 ±3.867b
Seaweed Ext., 500	49.082±1.347a	48.327±1.628ab	25.049±0.963a	24.823 ±0.908ab
Thiamine, 50	50.269±0.374a	50.161±1.121a	26.768 ±1.077a	26.390±1.524a

Values are given as mean± SD of three replicats. Means in columns by different letters are significantly different at P < 0.05 by Duncan's Multiple Rang t.

level of CAT and POD enzymes activity in plant tissue (Ortega-Ortiz *et al.*, 2007). Induction of CAT activity in the localized acquired resistance zone could play a protective role against possible oxidative damage (Dorey *et al.*, 1998). Consequently, a higher level of CAT activity in tomato shoot in response to biostimulants may be a pointer to the above observation. This has proved that besides its antifungal activity, CHT induces defense-related enzymes (Bautista-Banos *et al.*, 2006). A report found that when CHT was injected in date palm roots, it could induce increase in the activities of POD and PPO (El Hassni *et al.*, 2004).

3.2. Effect of biostimulants on antioxidant metabolites:

Data presented in Table(4) proved that application of all biostimulants, in particular 500 mg/l HA, significantly increased total soluble phenol, as compared with untreated control plants. The level of phenol increased by 20.244, 23.498, 22.960, 21.917 and 18.889, 23.768, 22.893, 21.985 mg/g fw in the shoot in both seasons in relation to CHI,HA, SE and Thi as compared with control plant (14.34 and 13.775).

Accumulation of secondary metabolites is another line of evidence for the role of biostimulants in disease resistance comes from their stimulatory effect on secondary metabolites production (Gundlach *et al.*, 1992). Our results indicated that biostimulants stimulated the accumulation of soluble phenolic compounds in tomato leaves. Specific phenolic compounds in different plant–pathogen interactions could open the way not only for revealing a pathogen attack but possibly also be integrated with an automatic plant stress resistance screening programs (Chaerle *et al.*, 2007). Recently, it was reported that the higher total phenolic content of the plant resulted in higher total antioxidant capacity (Cai *et al.*, 2004). Phenol structure has the ability to eliminate radical species (Ksouri *et al.*, 2008). It has been suggested that peroxidase could act as an efficient hydrogen peroxide scavenging system in plant vacuoles in the presence of phenolics and reduced ascorbate (Zancani and Nagy, 2000). Normally, phenol metabolism is activated in plants reacting to pathogens (Ellard-Ivery and Douglas, 1996). This is due not only to the mechanical role that phenolics play in cell walls, but also to their antifungal properties (Harborne, 1991). Yet total phenols have long been considered as important defence-related compounds whose levels are naturally high in resistant varieties of many crops (Gogoi *et al.*, 2001).

The data in Table (4) indicated that exogenous application of biostimulants increased significantly proline concentration in tomato shoot. Seaweed extract was the most effective in this concern, where it increased proline concentration from 8.695 and 8.806 to 17.627 and 18.944 mg/g fw in the first and second season, respectively. The accumulation of proline seemed faster and stronger under fungal stress. Delauney and Verma, (1993) found that proline accumulation is thought to function as a compatible osmolyte that stabilizes membranes and subcellular components, including the mitochondrial electron transport complex II. In addition, proline is proposed to scavenge free radicals (Siripornadulsil *et al.*, 2002) and to ameliorate shifts in redox potential by replenishment of the NADP+supply (Delauney & Verma, 1993 and Hare & Gress, 1997). In addition, proline accumulation seems to be greater when stimulated by applying low concentration of salicylic acid on tomato plants. This conclusion is in agreement with other studies, which documented the importance of biostimulants in pathogen-induced disease resistance and hypersensitive cell death (Delaney *et al.*, 1994 and Fabro *et al.*, 2004). Through the potentiation of oxidative burst, biostimulants can control both biotic and abiotic defense programs (Borsani *et al.*, 2001).

Regarding soluble sugars, the data presented in the same table clearly showed that all biostimulants application significantly increased total soluble sugars in tomato shoot in both growing season compared with untreated control plant. Chi was the

Table 4: soluble phenol (mg/g fw as gallic acid), Proline (mg/g fw), ascorbic acid (mg/g fw), soluble sugars (mg/g dw), and carotenoids (mg/g fw) concentration in tomato shoot as affected by biostimulants under natural infection of early blight after 100 days from transplanting.

Treatments (mg/l)	Compound									
	Phenol		Proline		Ascorbic acid		Soluble sugars		Total carotenoids	
	seasons									
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	14.346± 3.837c	13.775± 2.711c	8.790± 0.532c	8.806± 1.172c	12.166± 4.055c	12.110± 2.009d	17.711± 2.540e	17.093 ± 2.089e	0.184 ± 0.027b	0.218± 0.024b
Chitosan, 500	20.244± 2.428b	18.889± 4.187b	16.219± 1.357a	15.728± 1.164a	15.444 ± 2.693b	15.888± 1.387c	45.838± 1.087a	44.550± 4.912a	0.285 ± 0.140a	0.246 ± 0.028b
Humic acid, 500	23.498± 1.067a	23.768± 0.508a	16.696± 1.256a	16.702± 1.017a	16.444 ± 1.261ab	16.833± 0.881bc	22.531 ± 6.106d	21.352± 1.628d	0.309± 0.018a	0.281± 0.028b
Seaweed Ext., 500	22.960± 1.681a	22.893± 0.808a	17.627± 0.419a	17.530± 0.292a	18.944 ± 0.509a	18.555± 1.169a	36.987± 8.115b	35.393± 7.921b	0.319 ± 0.085a	0.384± 0.116a
Thiamine, 50	21.917± 0.649ab	21.985 ± 0.202a	12.608± 4.322b	11.387 ± 4.979b	17.833 ± 1.202ab	17.610± 0.961ab	30.589± 1.600c	30.125 ± 2.475c	0.369± 0.067a	0.414± 0.085a

Values are given as mean± SD of three replicats. Means in columns by different letters are significantly different at $P < 0.05$ by Duncan's Multiple Range Test.

most effective in this concern. Soluble sugars are involved in the responses to a number of stresses, and act as nutrient and metabolite signaling molecules that activate specific or hormonal – crosstalk transduction pathways, resulting in important modifications of gene expression (Couée *et al.*, 2006). Total soluble sugars in leaves treated with biostimulant significantly increased as compared to control plant. This increase could be related to the high photosynthetic pigments contents and then increased photosynthetic rate. These results indicated the relationship between sugar regulation and activaton of the systemic resistane. This increase might be due to the enhanced transcription of defence genes. Therefore, a sensor system based on the flux of carbohydrates regulates defence gene expression in plant cell (Blee and Anderson, 2000).

Regarding to AsA, it was observed from the data in Table (4) that AsA accumulation in tomato shoot due to biostimulants application. The most effective in this concern was seaweed extract here it increased the concentration from12.166 and 12.110 to 18.944 and 18.555 mg/g fw in the first and second season, respectively. The enhanced AsA concentration in biostimulants-sprayed plants may be due to de novo AsA biosynthesis and recycling of the oxidized forms of AsA, MDA and dehydroascorbate (Conklin and Barth, 2004). Under conditions of high AsA level as seen in biostimulant-sprayed plants, POD participated in the scavenging of hydrogen peroxide by the POD/phenolic/AsA system. In the present experiment, the increased ascorbate content in biostimulant spray may be sufficient to replenish reducing equivalents to phenoxy radicals thus explaining the increase in the phenolic compounds.

In conclusion, foliar application of biostimulants, in particular, Thi and SE enhanced the enzymatic and non-enzymatic antioxidants in tomato shoot infected by early blight disease, thus suppressing early blight infection-induced oxidative damage and enhancing early blight disease tolerance. The current limitation of the complete description of biostimulants signal transduction pathway in plants means that future studies are needed on the dissection of the complex network of elicitors and its involvement in plant defense against biotic and abiotic stresses using genetic, genomic and biochemical approaches.

REFERENCES

Abdalhadi, A.M.S. **2011**. Studies on the induction of systemic acquired resistance against some tomato fungal diseases by chemical activators. PhD thesis, Dept.of Agricultural Botany, Faculty of Agriculture, Saba Basha, Alexandria University.

Abdulgader, Y.A.T. **2011** Studies on the induction of systemic acquired resistance against some tomato fungal diseases by biotic inducers. PhD thesis, Dept.of Agricultural Botany, Faculty of Agriculture, Saba Basha, Alexandria University.

Abd-El-Kareem, F.; Abd-Alla, M.A. and El-Mohamedy, R.S.R. **2002**. Induced resistance in potato plants for controlling early blight disease under field condition. Egypt J. Appl. Sci., **17**: 51-66.

Abd-El-Kareem, F.; Abdallah, M.A; El-Gamal Nadia, G. and El-Mougy Nehal, S. **2004**a. Integrated control of Lupin root rot disease in solarized soil under greenhouse and field condition. Egypt J. Phytopathol., **32**: 49-63.

Abada, K.A.; Mostafa, S.H. and Hillal, M.R. **2008**. Effect of some chemical salts on suppressing the infection by early blight disease of tomato. *Egypt. J.Appl. Sci.*, **23** (20): 47-58.

Agrios, G.N. **2005**. Plant Pathology. 5th Ed. Acad. Press, San Diego, USA.

Asada, K. **2006**. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiology, **141(2)**:391-396.doi:10.1104/pp.106.082040.

Bates, L.S.; Waldren, R.P. and Teare, J.D. **1973**. Rapid determination of proline for water stress studies. Plant Soil **39**:205–207.

Bautista, B.S.; Hern, N.; Indez, L.; Bosquez, M.E. and Wil, C.L. **2003**. Effect of chitosan and plant extracts on growth of *Colletotrichum gloeosporioides*, anthracnose level and quality of papaya fruit. Crop Protection, **22**: 1087-1092.

Bautista-Banos, S.; Hernández-Lauzardo, A.N.; Velázquez-del, V.M.G.; Hernández-López, M.; Ait Barka, E.; Bosquez-Molina, E. and Wilson, C.L. **2006**. Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. Crop Prot., **25**: 108-118.

Bautista-Banos, S.; Hernandez-Lopez, M. and Bosquez-Molina, E. **2004**b. Growth inhibition of seleced ungi by chitosan and plant extracts. Mexican J. Phuytopathology, **22**:1678-186.

Beleid El-Moshaty, F.I.; Pike, S.M.; Novacky, A.J. and Seghal, O.P. **1993**. Lipid peroxidation and superoxide production in cowpea (*Vigna unguiculata*) leaves infected with tobacco ringspot virus or southern bean mosaic virus. Physiol Mol. Plant Pathol., **43**:109–119.

Blee, K.A. and Anderson, A.J. **2000**. Defense responses in plants to arbuscular mycorrhizal fungi. In: Podila GK, Douds DD, eds. Current advances in mycorrhiza research. Minnesota, USA. The American Phytopathological society, 27-44.

Blunden, G. **1991**. Agricultural uses of seaweeds and seaweed extracts. P 66-81. In: M. D Guiry and G Blunden (eds.). Seaweed resources in Europe: Uses and Potential. J. Wiley and Sons, Ltd. Chichester. UK.

Borsani, O.; Diaz, P.; Agius, M.F.; Valpuesta, V. and Monza, J. **2001**. Water stress generates an oxidative stress through the induction of a specific Cu/Zn superoxide dismutase in Lotus corniculatus leaves. Plant Sci., **161**:757–763. doi:10.1016/S0168-9452(01)00467-8.

Cai, Y.Z.; Luo, Q., Sun, M. and Corke, H. **2004**. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci., **74(17)**:2157–2184.

Cavalcanti, F.R.; Resende, M.L.; Lima, S.P.; Silveira, J.A. and Oliverira, J.T. **2007**. Activities of antioxidant enzymes and photosynthetic responses in tomato pre-treated by plant activators and inoculated by *Xanthomonas vesicatoria*. Physiol. Mol. Plant Pathol., **68(4-6)**: 198-208.

Cerkauskas, R. **2005**. Early blight. AVRDC, the world vegetable centre, www.avrdc.org.

Chaerle, L.; Lenk, S.; Hagenbeek, D.; Buschmann C. and Van Der Straeten, D. **2007**. Multicolor fluorescence imaging for early detection of the hypersensitive reaction to tobacco mosaic virus. J Plant Physiol., **164**:253–262.

Chen, Y.; De Nobili, M. and Aviad, T. **2004**. Stimulatory effect of humic substances on plant growth. In Soil Organic matter in sustainable agriculture (Eds F Magdoff, RR Weil) 103-130, Boca Raton, FL.

Chen, W.G.; Liu, X.; Chen, H.X. **2009**. Preparation of modified chitosan with quaternary ammonium salt. Textile Bioeng. Infor. Symp. Proc. 1, 226–230. 105–108.

Conklin, P.L. and Barth, G. **2004**. Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. Plant Cell Environment, **27**:959-970.

Couée, I.; Sulmon C.; Gouesbet, G. and EL Amrani, A. **2006**. Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. J. Exp. Bot., **57(3)**449- 459.

Croft, K.; Juttner, P.F. and Slusarenko, A.J. **1993**. Volatile products of the lipogenase pathway evolved from Phaseolus vulgaris L. leaves inoculated with *Pseudomonas syringae* PV *Phaseolicola*. Plant Physiol., **101**:13-24.

Delaney, T.P.; Uknes, S. B.; Vernooij, L.; Friedrich, K.; Weymann, D.; Negrotto, T.; Gaffney, M.; Gutt–Rella, H.; Kessmann, E.W and Ryals, J. **1994**. A central role of salicylic acid in plant disease resistance. Science, **266**: 1247–50.

Delauney, A.J. and Verma, D.P.S. **1993**. Proline biosynthesis and osmoregulation in plants. Plant J., **4**: 215–23.

Dorey, S.; Baillieul, F.; Saindrenan, P.; Fritig, B.; Kauffmann, S. **1998**. Tobacco Class I and II catalases are differentially expressed during elicitor-induced hypersensitive cell death and localized acquired resistance. Mol. Plant-Microbe Interact., **11**: 1102-1109.

EL Ghaouth, A.; Arul, J.; Asselin, A. and Benhamou, N. **1992c**. Antifungal activity of chitosan on post-harvest pathogen: inducition of morphological and cytological alterations in *rhizopus stolonifer*. Myol. Res., **96**:769-779.

El Hassni, M.; El Hadrami, A.; Daayf, F.; Barka, E.A. and El Hadrami, I. **2004**. Chitosan, antifungal product against *Fusarium oxysporum* f. sp. *albedinis* and elicitor of defence reactions in date palm roots. Phytopathol. Mediterr., **43**: 195-204.

EL-Ghamry, A.M.; Abd EL-Hai, K.M. and Ghoneem, K.M. **2009.** Amino and humic acids promote growth, yield and disease resistance of faba bean cultivated in clayey soil. Aus. J. Bascic and Applied Sciences, **3(2)**731-739.

Ellard-Ivery, M. and Douglas, C.J. **1996**. Role of jasmonates in the elicitor and wound-inducible expression of defense genes in parsley and transgenic tobacco. Plant Physiology, **112**, 183–192.

EL-Mohamedy, R.S.R. and Ahmed, M.A. **2009**. Effect of biofertilizers and humic acid on control of dry root rot disease and improvement yield quality of mandarin (*Citrus reticulate* Blanco). Research J. of Agriculture and Biol. Sci., **5(2)**: 127-137.

EL-Samra, I; Amer, M.A.; Abd-el-Hamid,M.R.; Kabeil, S.S;El-Alwani, A. M. **2011**. Chemical reaction in tomato plants in response to Abiotic elicitors Treatments. Marsland Press, J.Nature and Science,5402.

Fabro, G.; Pavet, I. V.; Szabados, I., and Alvarez, M.E. **2004**. Proline accumulation and ATPCS2 gene activation are induced by plant pathogen incompatible interactions in Arabidopsis. Americam Phytopath. Soc., **17**: 343-50.

Farouk, S.; Mosa, A.A.; Taha, A. A.; Heba, M.; Ibrahim1, A.M. and EL-Gahmery **2011**. Protective Effect of Humic acid and Chitosan on Radish (*Raphanus sativus*, L. var. *sativus*) Plants Subjected to Cadmium Stress. Journal of Stress Physiology & Biochemistry, Vol. 7 No. 2 2011, pp. 99-116 ISSN 1997-0838.

Farouk, S. **2009**. The roles of ascorbic acid and α-tocopherol in minimize of salt-induced wheat flag leaf senescence. J. Agric. Sci. Mansoura Univ., **34 (11)**: 10645 - 10661.

Farouk, S.; Ghoneem, K.M. and Abeer, A. A. **2008.** Induction and Expression of systematic resistance to downy mildew disease in cucumber plant by elicitors. Egyptian Journal of Phytopathology vol., **(1-2)**, 95-111.

Fraser, P.D. and Bramley, P.M. **2004**. The biosynthesis and nutritional uses of carotenoids. Progress in Lipid Research 43, 228–265. doi: 10.1016/J.Plipres.**2003**.10.002.

Frederikse, P.H.; Farnsworth, P. and Zigler, J.S.J. **1999**. Thiamine deficiency in vivo produces fiber cell degeneration in mouse lenses. Biochem-Biophys. Res. Commun., **258**:703-707.

Gao, J.G.; Xiao, Q.; Ding, L.P.; Chen, M.J.; Yin, l.; Li J.; Zhou, S.Y. and GY, H.E. **2008**. Differential responses of lipid peroxidation and antioxidants in *Alternanthera philoxeroides* and *Oryza sativa* subjected to drought stress. Plant Growth Regul., 56:89-95. doi:10.1007/s10725-008-9291-6.

Gogoi, R.; Singh, D.V. and Srivastava, D. **2001**. Phenols as a biochemical basis of resistane in wheat against karnal bunt. Plant Pathology, **50**:470-476.

Goncalves, J.F.; Becker, A.G.; Crgnelutti, D. L.A.; Tabaldi, L.B.; Pereira, V. Battisti, R.M.; Spanevello, V.M.; Morsch, Nicoloso, F.T. and Schetinger, M.R.C. **2007**. Cadmium toxicity causes oxidative stress and induces response of the antioxidant system in cucumber seedlings. Braz. J. Plant Physiol. **13(3)**:223-232.

Gundlach, H.; Müller, M.J.; Kutchan, T.M. and Zenk, M.H. **1992**. Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proc. Natl. Acad. Sci., USA. **89**: 2389-2393.

Hadwiger, L. and Loschke, D.C. **1981**. Molecular communication in host-parasite interactions: Hexosamine polymers (chitosan) as regular compounds in racespecific and other interactions. Phytopathology, **71**: 756-762.

Hameed, A.; Iqbal, N. and Malik, S.A. **2009**. Mannose-induced modulations in antioxidants, protease activity, lipid peroxidation, and total phenolics in etiolated wheat leaves. J. Plant Growth Regul., **28**:58–65.

Hameed, A.; Naseer, S.; Iqbal, T.; Syed, H. and Haq, M.A. **2008a**. Effects of NaCl salinity on seedling growth, senescence, catalase and protease activities in two wheat genotypes differing in salt tolerance. Pak. J. Bot., **40**:1043–1051.

Harborne, J.B. **1991**. Role of secondary metabolites in chemical defence mechanisms in plants. In: Bioactive Compounds from Plants. (D.J. Chadwick,, J. Marsh, ed.), John Wiley and Sons, Chichester, UK, 126–139.

Hare, P.D. and Cress, W.A. **1997**. Metabolics implications of stress-induced proline accumulation in plants. Plant Growth Regul **21**:79–102.

Harish Prashanth, K.V.; Dharmesh, S.M.; Jagannatha Rao, K.S.; Tharanathan, R.N. **2007.** Free radical-induced chitosan depolymerized products protect calf thymus DNA from oxidative damage. Carbohydr Res **342**:190–195. doi:10.1016/J.Carres.2006.11.010.

Jayaraj, J.; Wan, A.; Rahman, M. and Punja, Z.K. **2008**. Seaweed extract reduces foliar fungal diseases on carrot. Crop Protection Volume 27, Issue 10, October, Pages 1360-1366.

Ksouri, R.; Megdiche, W.; Falleh, H.; Trabelsi, N.; Boulaaba, M.; Samaoui, A. and Abdelly, C. **2008.** Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. CR Biol. Doi:10.1016/J.Crvi. 07.024.

Lamb, C. and Dixon, R.A. **1997**. The oxidative burst in plant disease resistance. Annu. Rev. Plant Physiol, Plant Mol. Biol., **48**:251-257.

Leuba, J.L. and Stossel, P. **1986**. Chitosan and other poluyamines: antifungal activity and interaction with biological membranes. In: Muzarelli R., Jeuniaux C, Graham GW (Eds), Chitin in Nature and Technology> Plenum Press, New York. USA. Pp 215-222.

Lin, C.C. and Kao, C.H. **2000**. Effect of NaCl stress on H2O2 metabolism in rice leaves. Plant Growth Regul., **30**:151–155.

Liu, J.; Tian, S.; Meng, X. and Xu, Y. **2007**. Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit. Postharvest Biol. Technol., **44**: 300-306.

Malolepsza, U. and Rozalaska, S. **2005**. Nitric oxide and hydrogen peroxide in tomato resistance. Nitric oxide modulates hydrogen peroxide level in O, hydroxyethylorutin-induced resistance to *Botrytis cinerea* in tomato. Plant Physiol. Bioche., **43**:623-635.

Melan, M.A.; Dong, X.; Endara, M.E.; Ausubel, K.R. and Peterman T.K. **1993**. An Arabidopsis thaliana lipogenase gene can be induced by pathogen, abscisic acid, and methyl jasmonate. Plant Physiol., **101**:441-450.

Morkunas, I. and Gemerek, J. **2007**. The possible involvement of peroxidase in defence of yellow lupin embryo axes against *Fusarium oxysporum*. J. Plant Physiol., **164**:497-506.

Momel, T.M. and Pemezny, K.L. **2006**. Florida plant disease management guide: Tomato. Florida Cooperation Extensive Service, Institute of Food and Agriculture Sciences, Gaineville, 32611.

Norris, D. and April M. **1991**. Methods for inducing resistance in plants using environmentally safe antioxidants. US Patent **5**:4-493.

Ortega-Ortiz, H.; Benavides-Mendoza, A.; Mendoza-Villarreal, R.; Ramírez- Rodríguez, H. and Romenus, K.D.A. **2007**. Enzymatic activity in tomato fruits as a response to chemical elicitors. J. Mex. Chem. Soc., **51**: 141-144.

Park, P.J.; JY J.E. and Kim, S.K. **2004**. Free radical scavenging activities of differently deacetylated chitosans using an ESR spectrometer. Carbohydr Polym **55**:17–22. doi:10.1016/J.Carbpol.,2003.05.002

Prapagdee, B.; Kotchadat, K.; Kumsopa, A. and Visarathanonth, N. **2007**. The role of chitosan in protection of soybean from sudden death syndrome caused by *Fusarium solani* f. sp. *glycines*. Bioresource Technology, **98(7)**: 1353-1358.

Rabea, E.; Badawy, M.T.; Stevens, C.; Smagghe, G. and Steurbaut, W. **2003**. Chitosan as antimicrobial agent: Applications and Mode of action. Biomacromolecules, **4(6)**: 1-8.

Radic,´ S.; Radic´-Stojkovic,´ M. and Pevalek, B.K. **2006**. Influence of NaCl and mannitol on peroxidase activity and lipid peroxidation in *Centaurea ragusina* L. roots and shoots. J. Plant Physiol., **163(12)**:1284–1292.

Radwan, D.E.M.; Fayez, K.A.; Mahmoud, S.Y.; Hamad, A. and Lu, G. **2006**. Salicylic acid alleviates growth inhibition and oxidative stress caused by zucchini yellow mosaic virus infection in *Cucurbita pepo* leaves. Physiol. Mol. Plant Pathol., **69(4–6)**:172–181.

Rao, M.V.; Paliyath, G.; Ormrod, D.P.; Murr, D.P. and Watkins, C.B. **1997**. Influence of salicylic acid on H2O2 production. oxidative stress, and H2O2 mtabolizing enzymes. Salicylic acid-mediated oxidative damage requires H2O2. Plant Physiol., **115**:137-149.

Reuveni, M. and Reuveni R. **1995**. Efficacy of foliar application of phosphate in controlling powdery mildew fungus on field grown wine graps: effects on cluster yield and peroxidase activity in berries. J. of Phyto., **143**:21-25.

Ryals, J.A.; Neuenschwander, U.H.; Willits, M.G.; Molina, A.; Steiner H.Y. and Hunt, M.D. **1996**. Systemic acquired resistance. Plant Cell, **8**:1809-1819.

Sadasivam, S. and Manickam, A. **1996**. Biochemical methods, Second eddition, New age international. India.

Scheuerell, S.J. and Mahaffee W.H. **2006**. Variability associated with suppression of Gray Mold (*Botrytis cinerea*) on geranium by foliar applications of nonaerated and aerated compost tea. Plant Disease, **90**:1201-1208.

Scheuerell, S.J. and Mahaffee, W.H. **2004**. Compost tea as a container medium drench for suppressing seedling damping off caused by *Pythium ultimum*. Phytopathology, **94**: 1156-1163.

Shao, H.B.; Liang, Z.S.; Shao, M.A. and Wang B.C. **2005**. Changes of some physiological and biochemical indices for soil water deficits among 10 wheat genotypes at seedlings stage. Colloids and Surfaces B: Biointerfaces, **42(2)**: 107-113.

Sharathchandra, R. G.; Shetty, N. P.; Amruthesh, K. N. and Shekar Shetty, H. **2004**. A chitosan formulation ElexaTM induces downy mildew disease resistance and growth promotion in pearl millet. Crop Protection, **23**: 881-888.

Shetty, N.P.; Ltken, R. H.; Haldrup, A.; Kema, G.H.; Collenge, D.P. and Jorgenson, H.J. **2007**. Role of hydrogen peroxide during the interaction between the hemibiotrophic fungal pathogen *Septoria tritici* and wheat. New Phytol., **174(3)**: 637-637.

Shinogi, T.; Suzuki, T.; Kurihara, T.; Narusaka, Y. and Park, P. **2003**. Microscopic detection of reactive oxygen species generation in the compatible and incompatible interaction of *Alternaria alternate* Japanese pear pathtype and host plant. J. Gen Pathol., **69**:7-16.

Singleton, V.L. and Rossi, J.A. **1965**. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am. J. Enol. Viticulture, **16**:144–158.

Siripornadulsil, S.; Traina, S.; Verma, D.P. and Sayre, R.T. **2002**. Molecular Mechanisms of proline–Mediated tolerance to toxic heavy metals in transgenic microalage. Plant Cell., **14**: 2837–47.

Sun, T.; Xie, W.M. and XU, P. X. **2004**. Superoxide anion scavenging actively of graft chitosan derivatives.Carb.Polym **58**:379-382. doi:10.1016./J.Carbpol.,06.042.

Sun, T.; Yao, Q.; Zhou, D.; Mao, F. **2008**. Antioxidant activity of N-carboxymethyl chitosan oligosaccharides. Bioorg. Med. Chem. Lett, **18**, 5774–5776.

Sun, T.; Zhou, D.; Xie, J.; Mao, F. **2006**. Preparation of chitosan oligomers and their antioxidant activity. Chem. Mater. Sci., **225 (3–4)**, 451–456.

Tyagi, M.; Kayastha, A.M. and Sinha, B. **2008**. The role of phenolics and peroxidase in resistance to *Alternaria triticina* in bread wheat (*Triticum aestivum* L.). J. Agron Crop Sci., **181**:29–34.

Velikova, V.; Yordanov, I. and Edreva, A. **2000**. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective roles of exogenous polyamines. Plant Sci., **151**:59–66.

Verkleij, F.N. **1992**. Seaweed extracts in agriculture and horticulture: a review. Biol. Agr. Hort., **8**:309-324.

Xu, Q.J.; Nian, Y.G.; JINXC; Yan, C.Z.; Liu, J. and Jiang, G.M. **2007**. Effects of chitosan on growth of an aquatic plant (*Hydrilla verticillata*) in polluted waters with different chemical oxygen demands. Chin. J. Environ. Sci., **19**:217–221

Yang, F.; Hu, J.; Li, J.; Wu, X. and Qian, Y. **2009**. Chitosan enhances leaf membrane stability and antioxidants enzyme activities in apple seedlings under drought stress. Plant Growth Regul., **58**:131-136.

Yang, F.; Jingjiang, H.u.; Jianlong, L.i.; Xiaoling, W.u. and Yurong, Q. **2009**. Chitosan enhances leaf membrane stability and antioxidant enzyme activities in apple seedlings under drought stress. Plant Growth Regul., **58**:131–136. DOI 10.1007/s10725-009-9361-4.

Zaky Wafaa, H.; Nada, M.G.A. and Hilal, A.A. **2006.** Evaluation of the efficincy of some environmentally safe means for controlling rust disease of anise (*Pimpinella anisum* L.) as important medicinal plant in Egypt. Egypt. J. Phytopathology, **34(2)** 103-119.

Zancani, M. and Nagy, G. **2000**. Phenol-dependent H_2O_2 breakdown by soybean root plasma membrane-bound peroxidase is regulated by ascorbate and thiols. J. plant Physiol., **156**:295-299.

Zhou, Z.; Wei, G.; Li, J.; Qian, Q. and Yu, J. **2004**. Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). Plant Sci., **167**:527–533.

الملخص العربى

إستحثاث المقاومة الجهازية لنبات الطماطم ضد مرض اللفحة المبكرة بواسطة المنشطات الطبيعية والفيتامينات

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إستحثاث مقاومة النبات ضد المسببات المرضية يعتمد علي شبكة متداخلة من الإشارات والتفاعلات الفسيولوجية. في الدراسة الحالية، استخدام المركبات الطبيعية كمعاملة بديلة أو مكملة للمبيدات الفطرية مفيدة جداً وبصفة خاصة نظراً لطول فترة جمع المحصول. استخدمت عدة مركبات طبيعية في الحقل تحت ظروف الإصابة الطبيعية لتقييم إمكانية استخدام المنشطات الطبيعية في مقاومة اللفحة المبكرة. دُرست تأثير المنشطات الطبيعية علي نمو النبات، أكسدة الدهون، نفاية الأغشية الخلوية، تراكم البرولين وفوق أكسيد الهيدروجين، وبعض المواد المضادة للأكسدة بالإضافة الي انزيم الكتاليز والبيروكسيديز في نباتات الطماطم تحت ظروف العدوى الطبيعية.

أجريت تجربتين حقليتين خلال الموسم الصيفي لعامي ٢٠٠٨و٢٠٠٩ بمزرعة قسم أمراض النبات – كلية الزراعة- جامعة المنصورة- مصر وذلك لدراسة تأثير رش المجموع الخضري لنبات الطماطم ببعض المنشطات الطبيعية (الكيتوزان- حمض الهيوميك- مستخلص الأعشاب البحرية- الثيامين) على استحثاث المقاومة وتقليل الإصابة بمرض اللفحة المبكرة. معاملة النباتات خارجيا بالمنشطات الطبيعية تحسن من قدرة نبات الطماطم علي تحمل مرض اللفحة المبكرة خلال انخفاض كمية فوق أكسيد الهيدروجين، نواتج أكسدة الدهون، معدل إرتشاح ونفاذية الاغشية الخلوية، مع زيادة نشاط انزيمات البيروكسيديز والكتاليز وعدد من المواد المضادة للاكسدة، مما يقلل من انتاج الشوارد الاوكسيجينية الحرة. كان الثيامين بتركيز ١٠٠ ملليجرام/لتر ومستخلص الاعشاب البحرية بتركيز ١٠٠٠ ملليجرام/لتر هم الأكثر تأثيراً في هذا الشأن. نخلص من النتائج إلي إمكانية استخدام المنشطات الطبيعية، خاصة، الثيامين ومستخلص الأعشاب البحرية في مقاومة مرض اللفحة المبكرة في الطماطم من خلال زيادة المواد المضادة للأكسدة، تقليل تراكم فوق أكسيد الهيدروجين، انخفاض أكسدة الدهون بالتالي تحسن خواص نفاذية الأغشية الخلوية. هذه المواد رخيصة الثمن، سهلة الحصول عليها واستعمالها وصديقة للبيئة.