Evaluation of Some Antioxidants against Tomato Early Blight Disease

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ABSTRACT

This work was carried out under greenhouse conditions during two successive seasons 2017 and 2018, at the Farm of Faculty of Agriculture, Menoufiya University, Shibin El-Kom to study the effect of three antioxidants i.e; salicylic acid, ascorbic acid and boric acid with two concentrations (15 and 30 mg/l) on tomato early blight disease incidence, disease severity, some growth characters, water relations and chemical compositions of tomato plants. All treatments of antioxidants significantly reduced disease incidence and disease severity, in as much as, increased significantly plant height, leaf area fresh and dry weight, total water content (TWC), bound water, free water, relative water content (RWC) and transpiration rate. Also, application of antioxidants increase in chlorophyll A, chlorophyll B, carotenoids, total carbohydrates, total sugars, protein and prolien on tomato plants. Salicylic acid (30 mg/l) was the best treatment followed by the (30 mgl/l) of ascorbic acid While, boric acid (15 mg/l) recorded the least effect on disease severity, disease incidence, growth characters, water relations parameters and chemical compositions.

Key words: Tomato, Early blight, Salicylic acid, Ascorbic acid, Boric acid, Growth, Water relations, Chemical compositions.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important solanaceous crops in Egypt and in the world because of its high contents of vitamin C as well as many chemical compounds and elements. Early blight disease caused by *Alternaria solani* (Ellis & Martin) Jones & Grout is considered as one of the most widespread tomato disease which can affect the foliage, stems and fruits. The disease is responsible for significant economic losses sustained by tomato or potato producers each year (Esmail zadeh *et al.,* 2008).

It is well known that using fungicides is considered as the shortest way to obtain efficient results for disease management. On the other hand, applications of fungicides have hazard effect on human and animal health. Therefore a more balanced, cost effective and eco-friendly approach must be implemented and adopted by farmers.

Antioxidants are resumed as alternative safety chemicals in this regard. According to Galal and Abdou (1996) some chemicals like antioxidants were reported as resistance inducers against plant disease.

Activating the natural defence mechanisms of the plant is one of the modern plant protection systems. Systemic acquired resistance (SAR) can be induced in various plants by chemical inducers.

Salicylic acid (SA) is an important signal molecule that plays a critical role in plant defence against pathogen invasion (Chaturredi and Shah, 2007). Application of salicylic acid has induced disease resistance on tomato plants against *Alternaria* leaf spot in glasshouse trials (Safari *et al.,* 2013). According to Ismail, Amal (2015) compost extract and chemicals i.e., Bion, salicylic acid, oxalic acid, Rubigan 12% and Topas could be used for controlling early blight of potato plants under field conditions.

Ascorbic acid, as foliar spray, reduced significantly early and late blight diseases incidence on tomato under greenhouse condition (Abde El-Kader *et al.,* 2012). According to Awadalla, Omyma (2008) five antioxidants (citric acid, salicylic acid, benzoic acid, ascorbic acid and sodium citrate) markedly inhibited the mycelial growth of *A. solani in vitro* and increased tomato resistance to early blight *in vivo*. Hossain *et al.* (2015) reported that potato seed treatment with 3% boric acid resulted in good control of common scab incidence with higher tuber yield.

The main objective of the present research was to evaluate the efficacy of three antioxidants (salicylic acid, ascorbic acid and boric acid) as resistance inducers against early blight disease on tomato.

MATERIALS AND METHODS

Isolation of Pathogen:

Samples of naturally infected leaves of tomato showing the early blight symptoms were collected from Vegetable Experiments Farm, Faculty of Agriculture, Shibin El-Kom, Egypt. Samples were subjected to isolation trials for the pathogenic fungus according to the method devised by Saad *et al.* (2014). The developed fungal colonies were purified on to Potato Dextrose Agar (PDA) medium by hyphal tip techniques. The purified cultures were identified depending on morphological and cultural characteristics (Ellis, 1971). Subcultures of the obtained isolate were kept on PDA slants and stored at 5°C until used.

Effect of antioxidants on the mycelial growth of *A. solani*:

This experiment was carried out using the method of Mate *et al.* (2005). Each antioxidant was dissolved in sterile distilled water and added separately to autoclaved potato dextrose agar (PDA), 10 ml of each concentration were added to 90 ml of PDA in flasks, before solidification at two different concentrations (15 and 30 mg/l) and using control without antioxidant. Four plates were used for each concentration (20 ml of PDA media in each plate). Plates were rotated gently to ensure equal distribution of the added antioxidants. After solidification of media each plate was inoculated with 5 mm (diameter) disc from 8 days old culture of *A. solani* in the center. Inoculated plates were incubated at 25°C until mycelial growth of the pathogenic fungus covered the surface of the medium in control treatment. The percentage of the radial growth inhibition was calculated as the formula given by Jayasinghe and Wijesundera (1995).

$$\text{Radial growth inhibition (\%)} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

Greenhouse experiment:

Antioxidants (salicylic acid, ascorbic acid and boric acid) were tested for controlling early blight disease on tomato plants under greenhouse condition at the Experimental Farm, Faculty of Agriculture, Menoufiya University, Shibin El-Kom, Egypt. Two seedlings (cv. Super strain B) 30-days-old, were transplanted in plastic pot (25-cm-diam.) containing 3 kg of clay-sand mixed soil previously disinfested by 5% formalin. Two weeks later the plants were sprayed with antioxidant at two concentrations (15 and 30 mg/l) (50 ml for each pot). One week after antioxidant treatments, each of tomato plants was inoculated with 20 ml of *A. solani* spore suspension containing 5×10^6 cfu / ml and kept under plastic bags for 48 hours to maintain high humidity (Abd El-Sayed, 2006). One week later, the plants received another spray with the tested antioxidants. Control plants were sprayed with the tested fungus only. Treatments were arranged in a completely randomized design. Five replicates were used for each treatment. Pots were watered as needed and fertilized with the recommended dose of N, P, K fertilizers. The disease incidence was recorded 40 days after transplanting, according to scale developed by Cohen *et al.* (1991) from 0 – 4 based on the leaf area infected as follows:

0 = No. leaf lesions, 1 = 25% or less of leaf area infected, 2 = 26 to 50%, 3 = 51 to 75% and 4 = 76 to 100% infected leaf area.

The disease severity (%) was recorded according to Ismail, (2015) formula:

$$\text{Disease severity} = \frac{\Sigma (N \times V)}{G\,N} \times 100\%$$

N = Total number of the infected leaves,
V = Numerical grade,
G = Higher degree in the category.

Also, Plant samples were taken to determine the following parameters:

1. Vegetative growth characters: Root length (cm), plant height (cm), leaf area per plant (cm^2 / plant). (Fladung and Ritter, 1991), fresh and dry weight of hole plant (g) (Plant materials were dried in an electric oven at 70°C for 72 hours).
2. Water relations: Total water content (TWC %), free and bound water (Gosev, 1960 and Kreeb, 1990), relative water content (RWC %) (Barrs and Weatherley, 1962), osmotic pressure (Gosev, 1960), transpiration rate (Kreeb, 1990).
3. Photosynthetic pigments: The photosynthetic pigments were extracted from fresh leaf sample (fourth upper leaf) by 85 % acetone according to the method described by Wettestein's formula in A.O.A.C., 1995.
4. Chemical analysis: Total carbohydrates and total sugars were determined using the phenol sulfuric acid method as described by A.O.A.C. (1995). Proline concentration was measured according the method of Bates *et al*. (1973).

Statistical analysis:

All experiments were performed twice. The obtained data were subjected to analysis of variance (ANOVA) using Costat software, version 6.4 (2008). Duncan's multiple range test (DMRT) at $p < 0.05$ level was used for means separation (Gomez and Gomez (1984).

RESULTS

Data presented in Table (1) show that application of antioxidant; salicylic acid, ascorbic acid, boric acid at two different concentrations (15, 30 mg/l) caused significant reduction on liner growth of *Alternaria solani in vitro*. The heights reduction was observed by salicylic acid 30 mg/l (81.11%) followed by ascorbic acid 30 mg/l (76.66%). On the other hand boric acid 15 mg/l gave the lowest effect on *A. solani* linear growth (51.11%).

Presented data in Table (2 and 3) reveal that disease incidence and disease severity of early blight disease were reduced significantly in the two seasons 2017, 2018 when plants were sprayed with antioxidants compared with check treatment. Salicylic acid 30 mg/l had the greatest inhibitory effect on early blight disease appearance in the two seasons (75.85 and 76.26% reduction in disease incidence at 2017 and 2018, respectively) and in disease severity (60.97 and 63.04% reduction at 2017 and 2018, respectively).

Table 1: Effect of two concentrations of three antioxidants on the linear growth of *Alternaria solani in vitro*.

Treatments	Concentrations (mg /l)	Linear growth (mm)	Reduction (%)
Salicylic acid	15	27[e]	70.00
	30	17[g]	81.11
Ascorbic acid	15	30[d]	66.66
	30	21[f]	76.66
Boric acid	15	44[b]	51.11
	30	36[c]	60.00
Control	–	90[a]	–

Values with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range Test.

Table 2: Effect of two concentrations of three antioxidants on early blight disease incidence on tomato plants under greenhouse condition during 2017 and 2018 growing seasons.

Treatments	Concentrations (mg/l)	2017 season		2018 season	
		Disease incidence	Reduction (%)	Disease incidence	Reduction (%)
Salicylic acid	15	1.05[e]	50.70	0.95[e]	52.02
	30	0.52[g]	75.58	0.47[g]	76.26
Ascorbic acid	15	1.32[d]	38.02	1.27[d]	35.85
	30	0.75[f]	64.78	0.72[f]	63.63
Boric acid	15	1.60[b]	24.88	1.57[b]	20.70
	30	1.42[c]	33.33	1.40[c]	29.29
Control (*A. solani*)	–	2.13[a]	–	1.98[a]	–

Values with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range Test.

Table 3: Effect of two concentrations of three antioxidants on early blight disease severity on tomato plants under greenhouse condition during 2017 and 2018 growing seasons.

Treatments	Concentrations (mg/l)	2017 season		2018 season	
		Disease severity	Reduction (%)	Disease severity	Reduction (%)
Salicylic acid	15	23.9[e]	55.15	22.5[e]	55.53
	30	20.8[g]	60.97	18.7[g]	63.04
Ascorbic acid	15	28.5[d]	46.52	26.7[d]	47.23
	30	22.2[f]	58.34	20.7[f]	59.09
Boric acid	15	36.9[b]	30.76	35.5[b]	29.84
	30	32.4[c]	39.21	31.4[c]	37.94
Control (*A. solani*)	–	53.3[a]	–	50.6[a]	–

Values with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range Test.

While the lowest inhibitory effect on disease appearance was observed by boric acid 15mg/l (24.88 and 20.70% reduction in disease incidence and in disease severity (30.76 and 29.84% reduction at 2017 and 2018 seasons).

Growth characters:

Data illustrated at Table (4) showed that, all treatments caused a significant increase in all growth characters, i.e. root length, plant height, leaf area, fresh and dry weight .The maximum increase in all growth characters were recorded at the second level of salicylic acid treatment increased by a bought 8.3, 2.5, 18.68, 1.35 and 0.12 % over the control plants respectively, at the first season compared to control plants. The same trend was observed in the second season.

Water relations:

Data in Table (5) cleared that, TWC, free water, bound water and RWC were increased at the all levels of antioxidants under test as this sequences salicylic acid, ascorbic acid and boric acid. Meanwhile, the osmotic pressure and the transpiration rate were decreased at the same levels of antioxidants. The second level of salicylic acid recorded a highest increase in TWC, free water, bound water and RWC in tomato leaves. The increase rate over the control plants was a bought 7.02, 0.49, 6.53 and 3.94 %, respectively, when compared with the control plants. Similar results were obtained in the second season.

As shown in Table(6) during the two growing seasons of 2016 and 2017 all applied treatments of antioxidants led to significant increase of chlorophyll a, chlorophyll b, carotenoids, total carbohydrates, total sugars, protein and prolien in leaves of treated tomato plants. There was remarkable highest increase in chemical composition and photosynthetic pigments at the second level of salicylic acid followed by the second level of ascorbic acid.

Table 4: Effect of some antioxidants on growth characters of tomato plants grown under greenhouse and artificially infected with *Alternaria solani* at two growing seasons 2017 and 2018.

Treatments \ Characters		Root length (cm)	Plant height (cm)	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)
	mg/l			**Season 2017**		
Control	0	26.3	58.9	147.40	31.76	2.74
Alternaria solani	0	21.8	49.2	122.83	26.53	2.29
Salicylic acid	15	32.8	63.3	166.25	34.14	2.94
Salicylic acid	30	34.6	61.4	166.08	33.11	2.86
Ascorbic acid	15	28.4	62.4	157.08	33.65	2.90
Ascorbic acid	30	33.9	59.6	161.76	32.14	2.77
Boric acid	15	29.1	57.7	150.16	31.12	2.68
Boric acid	30	29.5	59.2	153.45	31.92	2.75
LSD at 5%		0.0009	1.058	0.129	0.039	0.032
				Season 2018		
Control	0	27.0	60.5	146.43	32.95	2.84
Alternaria solani	0	22.3	50.3	121.89	27.47	2.37
Salcylic acid	15	33.1	63.9	160.34	36.69	3.16
Salcylic acid	30	35.3	62.6	159.40	36.37	3.14
Ascorbic acid	15	29.1	63.8	155.31	35.06	3.02
Ascorbic acid	30	34.6	60.8	155.09	35.39	3.05
Boric acid	15	29.2	57.9	144.83	33.22	2.86
Boric acid	30	30.2	60.6	150.06	33.98	2.93
LSD at 5%		0.0006	1.090	0.127	0.046	0.038

Table 5: Effect of some antioxidants on water relations of tomato plants grown under artificial infection with *Alternaria solani* at two growing seasons 2017 and 2018.

Treatments \ Characters		TWC (%)	Free water (%)	Bound water (%)	RWC (%)	O.P. C.S. (bar)	Trans rate (mg/g fw.h)
	mg/l			Season 2017			
Control	0	81.09	11.65	69.44	75.08	2.36	0.027
Alternaria solani	0	78.47	9.73	68.74	72.66	2.39	0.028
Salicylic acid	15	86.14	12.52	73.62	77.26	2.28	0.025
Salicylic acid	30	88.11	12.14	75.97	79.02	2.23	0.023
Ascorbic acid	15	84.89	12.34	72.55	77.88	2.24	0.025
Ascorbic acid	30	87.59	11.79	75.80	78.56	2.23	0.023
Boric acid	15	78.91	11.41	67.50	74.80	2.29	0.027
Boric acid	30	79.73	11.71	68.02	75.57	2.29	0.027
LSD at 5%		0.0009	0.437	1.629	0.495	1.994	0.0005
				Season 2018			
Control	0	81.02	11.62	69.40	74.79	2.37	0.026
Alternaria solani	0	76.93	9.56	67.37	71.22	2.41	0.022
Salcylic acid	15	85.25	12.45	72.80	76.40	2.29	0.028
Salcylic acid	30	87.22	12.08	75.14	78.16	2.24	0.027
Ascorbic acid	15	83.97	12.24	71.73	77.00	2.24	0.027
Ascorbic acid	30	85.86	11.69	74.27	76.97	2.24	0.026
Boric acid	15	78.07	11.39	66.68	73.89	2.30	0.025
Boric acid	30	78.86	11.66	67.20	74.66	2.30	0.026
LSD at 5%		0.0006	0.419	1.491	0.479	1.982	0.0006

Table 6: Effect of some antioxidants on chemicals constituents of leaves of tomato plants grown under artificial infection with *Alternaria solani* at two growing seasons 2017 and 2018.

Treatments \ Characters		Chl. A. (mg /g dwt)	Chl. B. (mg /g dwt)	Carote-noides (mg /g dwt)	Total Carbohy-drates (mg /g dwt)	Total sugars (mg /g dwt)	Protein (%)	Proline (µg / /g dwt)
	mg/l				Season 2017			
Control	0	3.18	1.14	1.29	0.824	0.015	24.27	238.31
Alternaria solani	0	3.08	1.02	1.21	0.815	0.012	23.81	261.04
Salicylic acid	15	3.27	1.23	1.32	0.841	0.017	24.97	282.41
Salicylic acid	30	3.35	1.33	1.38	0.863	0.017	25.72	300.59
Ascorbic acid	15	3.30	1.25	1.34	0.849	0.017	24.92	271.46
Ascorbic acid	30	3.33	1.26	1.35	0.855	0.017	25.46	292.31
Boric acid	15	3.17	1.14	1.27	0.828	0.015	24.44	264.66
Boric acid	30	3.20	1.15	1.28	0.833	0.016	24.39	266.24
LSD at 5%		0.088	0.116	0.057	0.008	0.0007	0.289	3.474
					Season 2018			
Control	0	3.17	1.14	1.30	0.821	0.015	24.31	241.73
Alternaria solani	0	3.02	1.00	1.18	0.799	0.013	23.79	250.85
Salcylic acid	15	3.24	1.22	1.31	0.832	0.017	24.93	288.92
Salcylic acid	30	3.31	1.32	1.36	0.854	0.017	25.67	297.01
Ascorbic acid	15	3.26	1.24	1.32	0.839	0.017	24.88	281.01
Ascorbic acid	30	3.27	1.23	1.34	0.838	0.017	25.56	283.30
Boric acid	15	3.13	1.13	1.25	0.818	0.016	24.38	280.94
Boric acid	30	3.16	1.14	1.26	0.823	0.016	24.35	275.82
LSD at 5%		0.079	0.121	0.060	0.009	0.0004	0.267	2.093

DISCUSSION

Early blight disease caused by *A. solani* is one of most serious foliar diseases causing large yield losses in tomato plants especially in plastic houses (Jagadeesh and Jagadeesh, 2009). Fungicides could successfully control plant diseases; however, they have many hazardous effects on animal environment and human.

Plant resistance inducers are among the most important alternative methods for controlling plant diseases which are safe and rapidly biodegradable (Ragab *et al.,* 2009). Application of ascorbic acid reduced significantly early blight disease incidence of potato (El-Gamal *et al.,* 2007). Also, spray application of ascorbic acid to cucumber and pepper plants had a good effect on reducing the powdery and downy mildew disease severity (Abd El-Kader *et al.,* 2012).

Salicylic acid plays an important role in induction resistance of plants to pathogens. According to Vallad and Goodman (2004) salicylic acid can induce accumulation of pathogenesis-related proteins (PRP) and lead to reduced incidence of several diseases on many crops. Application of salicylic acid and ascorbic acid elicit production of tomatin (phytoalexin) in leaves and stems of tomato plants which is toxic to *A. solani* (Awadella, Omyma 2008). The results obtained by Ismail *et al.* (1988) showed that, salicylic acid had an inhibitory effect on germ tube length and spores germination of *Fusarium oxysporum* f.sp. *lycopersici* and *Aspergillus fumigatus*.

Exogenous application of salicylic acid delayed the development of *Botrytis cinerea* on tobacco leaves (Murphy *et al.,* 2000).

The reduction of early blight disease on tomato plants which were treated with the antioxidants (salicylic acid, ascorbic acid and boric acid), improved growth characters, water relations, photosynthetic pigments and chemical constituents. Mady (2009) found that, different applied treatments (salicylic acid) significantly increased all studied growth parameters as number of branches and leaves per plant, leaf area per plant and leaves dry weight as well.

Sanaa *et al.* (2006) and Mady (2009) reported that, applied of salicylic acid or vitamin E obviously increased photosynthetic pigments, total carbohydrates and crude protein concentrations in leaves of treated plants as compared with those of untreated ones on bean and tomato plants. Application of salicylic acid to plants has shown a variety of biological responses.

The resistance of the disease depends on the chemical activity of the plant cell and the activity of the formation of particular protein or a particular enzyme. It's worth mentioning that all the metabolic activity in the plant depends on the good water balance in the cell which due to cell bloating, opening and closing of stomata and the hardness of the cell wall.

Enzyme activities such as amylase and nitrate reductase were increased by salicylic acid application (Sharma *et al.,* 1986 and Chen *et al.,* 1993). On the other hand, SA showed synergetic effect with auxin and gibberellins (Datta and Nanda, 1985 and Sanaa *et al.,* 2006). Moreover, in a number of species SA promoted flowering in combination with other plant growth regulators such as kinetin, indole acetic acid and gibberellins (Singh, 1984 and Shehata *et al.,* 2000). Application of SA induced changeable in endogenous phytohormones of tomato and other plants (Raskin, 1992 and Waffaa *et al.,* 1996).

In conclusion, the obtained results showed the antifungal effect of antioxidants and their value in controlling early blight disease and improved growth characters of treated tomato plants. So antioxidants could be recommended to control early blight disease and reduces the hazard of fungicides.

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الملخص العربي

تقييم بعض مضادات الأكسدة ضد مرض اللفحة المبكرة في الطماطم

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تم إجراء الدراسة تحت ظروف الصوبة خلال موسمى النمو ٢٠١٧ – ٢٠١٨ فى مزرعة كلية الزراعة – جامعة المنوفية لدراسة تأثير بعض مضادات الأكسدة (حمض السليسليك – حمض الأسكوربك – حمض البوريك) بتركيزين مُختلفين (١٥ – ٣٠ ملليجرام / لتر) على نسبة الاصابة و شدة الاصابة بمرض اللفحة المُبكرة على الطماطم. بلإضافة لدراسة بعض صفات النمو والعلاقات المائية والمُكونات الكيميائية لنبات الطماطم. وقد أوضحت النتائج أن جميع المُعاملات أدت لخفض نسبة وشدة الإصابة بالمرض بالتوازى مع زيادة فى صفات النمو (ارتفاع النبات – مساحة الورقة – الوزن الجاف للنبات). كذلك تم تسجيل زيادة فى العلاقات المائية (المحتوى المائى الكلي – الماء المُرتبط – المُحتوى المائي النسبي – الماء الحُر)، وأيضاً زيادة في المُكونات الكيميائية (كلوروفيل أ – كلوروفيل ب – الكربوهيدرات الكلية – السكريات الكلية – البرولين – كاروتونيدات – البروتين). وكانت أفضل المُعاملات هي حمض السليسليك بتركيز ٣٠ ملليجرام / لتر، يليه حمض الأسكوربك بتركيز ٣٠ ملليجرام/ لتر، بينما سجل حمض البوريك تركيز ١٥ ملليجرام / لتر أقل المُعاملات فاعلية.