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Chemical control of tomato early blight caused by *Alternaria solani* using certain fungicides and chemical inducers

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Abstract

Eight isolates were isolated from tomato plants identified as *Alternaria solani* were involved in pathogenicity tests which resulted in definite symptoms of early blight disease of tomatoes. Four antioxidant compounds were tested against both aggressive and non-aggressive isolates, salicylic acid showed high efficacy in reducing growth of *A. solani* even as linear growth *in vitro* or as disease severity *in vivo* followed by citric acid and catechol respectively, while, ascorbic acid showed lowest effect. On the other hand, laboratory and greenhouse experiments were carried out to evaluated four fungicides for their inhibitory effect on the growth of *A. solani* aggressive and non-aggressive isolates. Bellis fungicide recorded the best result in controlling the disease either *in vitro* or *in vivo* as compared to other fungicides and control. Amistar-top and luna experience exhibited intermediate effect on the fungus isolate, and the lest order was achieved by folio- gold fungicide. In general, fungicides were more effective in controlling the disease than the tested antioxidants but, environmental pollution and hazardous effect, on human health must be considered.

Keywords: *Alternaria solani*, fungicides, antioxidants, tomato, early blight.

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1. Introduction

Tomato (*Lycopersicon esculentum*) is one of the vital solanaceous vegetable crops of global importance grown in Egypt. The cultivated area of tomato since 2009 to 2014 growing seasons reached 499.536 feddan in old and newly reclaimed lands, which produced about 7.964.997 tons (FAO, 2015). Tomato ranks next to the potato crop and ranks the first among the processing crops in the world. *Alternaria solani* (Ellis and Martin) Jones and Grout is the most important pathogen causing severe early blight disease every year in tomato. The disease causes a drastically reduction in tomato fruits quantity and quality. Symptoms of the disease are characterized by brown to dark brown colored necrotic spots (Mayee et al., 1986). Due to this disease, about 80% yield loss was recorded in experimental field and severity varies from 15-90% (Pandey et al., 2003). Most currently grown tomato cultivars are susceptible to early blight at various degrees; consequently, foliar fungicides are used frequently to manage this disease. The most effective early blight control measure consists of excessive fungicides applications starting early in the growing season before the first symptom appear (Pscheidt et al., 1988). Primary methods of controlling early blight include preventing long periods of wetness on the leaf surface, cultural scouting, sanitation and development of the host plant resistance with the application of fungicides (Kumar et al., 2013). Chemical inducers of plant resistance possess quite different modes of action as compared to synthetic biocides as they have no direct toxicity to pathogens, plants and animals, no negative effects on plant growth, yield development, broad spectrum of defense, long lasting protection and low economical cost for farmers and good profit for producers (Horsfield et al., 2010; Kessmann et al., 1994). Salicylic acid (SA) plays an important role in plant defense, and is well documented for dicotyledonous plants, where it's required for basal resistance against pathogens as well as for the inducible defense mechanism and systemic acquired resistance (SAR) which confers resistance against a broad-spectrum of pathogens (Chaturvedi et al., 2007). The effect of five antioxidants (citric acid, salicylic acid, benzoic acid, ascorbic acid and sodium citrate) on the resistance of tomato plants (*Lycopersicon esculentum* Mill.) to early blight disease incited by *Alternaria solani* was investigated *in vitro* and *in vivo* (Awadalla et al., 2008). Therefore, the present study was aimed to determine the efficacy of different fungicides and antioxidant doses against *Alternaria* early blight of tomato.

2. Materials and methods

2.1 Isolation and identification of the causal organism

Leaves of diseased tomato plants showing typical early blight symptoms were collected from different localities of Luxor, Qena, Sohag, Assiut and Minia governorates, Egypt during 2013 growing season. Samples were washed using tap-water surface sterilized with 0.5% sodium hypochlorite solution for two minutes then washed three times in sterilized distilled water. Samples were then dried between two layers of sterilized filter paper to remove the excess water. The sterilized spotted leaf tissues were cut with adjacent healthy tissues using a sterile scalpel and placed on plain agar medium in Petri-dishes. Inoculated dishes were incubated at 27°C for 9 days. Hyphal tips from the outer

ends of the growing colonies were transferred to plates of potato dextrose agar (PDA) medium and incubated at 2٧°C. Pure cultures were obtained for each of the isolated fungi using the single spore technique according to (Hansen et al., 1926) and/or hyphal tip technique according to (Brown et al., 1924). The purified fungi were identified at Plant Pathology Research Institute, Agricultural Research Center according to their morphological characters using the description of (Ellis et al., 1993). Stock cultures were maintained on PDA slants and stored in a refrigerator at 7±2°C.

2.2 Pathogenicity tests

Tomato transplants (Super strain B) were grown in plastic pots filled with sterilized soil. Thirty days after transplanting, plants were sprayed with sterile distilled water before inoculation then covered with polyethylene bags for 24 hours. Spores and mycelial suspensions for the eight isolates obtained from isolation and identified as *A. solani* were prepared in sterile distilled water from nine days old cultures. The spore suspensions were spread and swabbed with moist cotton onto leaves after being scratched using carborundum. Such inoculated plants were again covered with polyethylene bags. After 24 hours of incubation, polyethylene bags were removed and the plants were kept in greenhouse. Control was maintained by spraying the plants with only sterile distilled water. Disease severity was recorded after 30 days of inoculation. Re-isolation was made from infected plants and the cultures thus obtained were compared with original cultures to confirm the identity and the pathogenicity of the pathogens. The two extreme aggressive and non-aggressive isolates were selected for further experiments.

2.3 Disease assessment

Scale from 0 to 5 according to (Mayee et al., 1986) was used to assess the disease where: 0 = No symptoms on the leaf. 1 = 0-5 percent leaf area infected and covered by spots, no petioles and branches. 2 = 6-20 percent leaf area infected and covered by spots, some spots on petioles. 3 = 21-40 percent leaf area infected and covered by spots, spots also seen on Petioles and branches. 4 = 41-70 percent leaf area infected and covered by spots, spots also seen on Petioles, branches and stems. 5 = 71-100 percent leaf area infected and covered by spots, spots also seen on petioles, branches, stems and fruits. Disease severity was calculated according to the formula:

$$\text{Disease severity (\%)} = \sum [(n \times V) / 5xN)] \times 100$$

Where: n = Number of infected leaves in each category. V = Numerical values of infection categories. N = Total number of leaves examined. 5 = Constant, highest numerical value.

2.4 Effect of chemical inducers on *A. solani*

2.4.1 *In vitro*

Different concentrations (500, 1000, 1500, 2000, 2500 and 3000 ppm) of four chemical inducers *i.e.* ascorbic acid, catechol, citric acid and salicylic acid were incorporated in 100 ml PDA medium and poured in 9 cm Petri dishes.

A five mm diameter agar disc containing fungal mycelium growth of the two tested fungi was transferred to the test medium (Three plates for each concentration and control). Then plates were incubated at 27±1°C in a growth chamber. Colony diameter was observed daily until control Petri dishes were covered with the fungal growth and measured as percentage reduction of linear growth of pathogenic fungi comparing with control using this formula:

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

If in a trial 30% of introduced weevils were found in the EO portion of the disc, it means that percent repellency by the oil was 70%. A total of 20 trials were carried out and percent repellency due to the essential oil and ethanol was recorded accordingly.

2.4.2 *In vivo*

To study the effect of ascorbic acid, catechol, citric acid and salicylic acid on the disease severity of *A. solani* isolates, plastic pots (25 cm in diameter) were filled with sterilized soil. Tomato transplants (super strain B) were transplanted at the rate of 3 transplants /pot. This experiment was carried out under greenhouse conditions with three replicates for each particular treatment. After 3 weeks from transplanting, tomato plants were sprayed with the tested chemical inducers. Plants which were sprayed with distilled water before inoculation served as control. After seven days, plants were inoculated with the suspensions of *A. solani* isolates (10^6cfu/ml). The sprayed plants were covered with polyethylene bags for 24 hours under greenhouse conditions. After 30 days from inoculation, disease severity was calculated as mentioned before. The tested inducers were sprayed with the best concentration for each particular compound which gave the best result in reducing linear growth o *A. solani* isolates *in vitro*.

2.5 Effect of fungicides on *A. solani*

2.5.1 *In vitro*

The effect of different concentrations (5, 10, 25, 50, 100, 150, 200 and 300 ppm active ingredient) of four fungicides; Bellis 38% WG (25.2% Boscalid + 12.8% Pyraclostrobin), Amistar-top 32.5% SC (20% Azoxystrobin + 12.5% Difenoconazole), Folio gold 53.75% SC (3.75% Metalaxyl M + 50% Chlorothalonil) and Luna experience 40% SC (20% Fluopyram + 20% Tebuconazol) was tested on the linear growth of *A. solani* isolates on PDA media. The different concentrations of each fungicide (according to the active ingredient) were suspended and added to PDA medium before solidification. Then media containing fungicides were poured in Petri dishes (9 cm in diameter) and three Petri dishes were used for each concentration alone. The dishes were inoculated in the center with equal discs (5 mm in diameter) of 9 days old culture of *A. solani* isolates and incubated as mentioned before. Colony diameter was observed daily until control Petri dishes were covered with the fungal growth and measured as percentage reduction of linear growth of pathogenic fungi comparing with control using this formula:

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

2.5.2 *In vivo*

To study the effect of the tested fungicides on the disease severity, plastic pots (25 cm in diameter) were filled with sterilized soil. Tomato seedlings (super strain B) were transplanted with 3 transplants /pot. This experiment was carried out under greenhouse conditions with three replicates for each particular treatment. After 3 weeks from transplanting, tomato plants were sprayed with the tested fungicides. Plants which were sprayed with distilled water before inoculation served as control. After seven days, plants were inoculated with the suspensions of *A. solani* isolates (10^6cfu/ml). The sprayed plants were covered with polyethylene bags for 24 hours under greenhouse conditions. After 30 days from inoculation, disease severity was calculated as mentioned before. The tested fungicides were sprayed with the best concentration for each particular compound which gave the best result in reducing linear growth *in vitro* of *A. solani* isolates.

2.6 Statistical analysis

Analysis of variance of the data was carried out on the mean values of the tested treatments according to the procedures described by Gomez and Gomez (1984). The least significant difference (LSD) at 5% probability was used for testing of significance of the differences among the mean values of the tested treatments for each character.

3. Results and Discussion

3.1 Pathogenicity tests

Data presented in Table (1) showed that all the tested isolates proved to be pathogenic to the tested tomato plants (Super strain B), causing symptoms of early blight which, first, appear as small irregular to circular dark brown spots on the lower (older) leaves which turn yellow and die, in comparison with the control. In this regard, *Alternariasolani* isolate Assiut-A3 was significantly the most aggressive isolate recording the highest disease severity value (57.38), followed by Minia-M (50.65), Assiut-A2 (48.46), Assiut-A1(34.66), Sohag-S (30.44), Luxor-L2 (27.3) and Luxor-L1 (23.3), respectively. While, Qena-Q (22.6) recorded the lowest disease severity. Hence, the isolates Assiut-A3(most aggressive) and Qena-Q (least aggressive) were chosen for the following experiments.

Table 1: Pathogenicity tests of *Alternaria solani* isolates on susceptible tomato plants (Super strain B) under greenhouse conditions.

Isolate No.	Governorate	Disease severity (%)
1	Luxor-L1	23.3
2	Luxor-L2	27.30
3	Qena-Q	22.60
4	Sohag-S	30.44
5	Assiut-A1	34.66
6	Assiut-A2	48.46
7	Assiut-A3	57.38
8	Minia-M	50.65
Control		00.0
L.S.D at .05%		3.07

3.2 Effect of chemical inducers on *A.solani*

3.2.1 *In vitro*

Four chemical inducers *i.e.* ascorbic acid, catechol, citric acid and salicylic acid were tested for their effect on the mycelial growth of *Alternaria solani* isolates.

Data in Table (2), showed that, all tested concentrations (500, 1000, 1500, 2000,

2500 and 3000 ppm) of four chemical inducers reduced the growth of *A. solani* isolates as compared with control. Salicylic acid gave the highest *A. solani* isolates linear growth decrease, followed by citric acid and catechol respectively. While, ascorbic acid gave the lowest effect.

Table 2: Effect of different concentrations of some chemical inducers on the linear growth of *A. solani* isolates.

Antioxidant	Conc. (ppm)	Mycelial growth inhibition (%)	
		Aggressive	Non Aggressive
Ascorbic acid	500	20.6	55.6
	1000	36.3	63.6
	1500	48.3	75.6
	2000	55.6	85.3
	2500	70.3	92.6
	3000	79.3	100
Salicylic acid	500	36.6	63.3
	1000	49.3	74.3
	1500	59.6	91.3
	2000	75.3	100
	2500	88.3	100
	3000	100	100
Citric acid	500	32.6	53.3
	1000	45.6	61.3
	1500	57.3	72.6
	2000	70.6	84.6
	2500	87.3	100
	3000	100	100
Catechol	500	27.3	45.6
	1000	35.3	56.6
	1500	46.6	66.6
	2000	65.3	78.6
	2500	84.3	90.3
	3000	100	100
Control		0.00	0.00
Treatment A		2.59	1.11
Conc B		1.45	1.05
A×B		3.25	2.36

3.2.2 *In vivo*

Four chemical inducers *i.e.* ascorbic acid, catechol, citric acid and salicylic acid were tested for their effect on the disease severity at certain concentrations under greenhouse conditions. Data in Table (3) showed that, all tested concentrations of the tested chemical inducers resulted a significantly reduction of early blight disease severity, as compared with control. At all concentrations, salicylic acid was the most effective, followed by citric acid and catechol, respectively. While, ascorbic acid was less effective.

3.3 Fungicides

3.3.1 *In vitro*

The effects of different concentrations (5, 10, 25, 50, 100, 150, 200 and 300 ppm active ingredient) of the four fungicides (Bellis 38% WG, Amistar-top 32.5% SC, Folio gold 53.75% SC and Luna experience 40% SC) were tested on the

linear growth of *A. solani* isolates on PDA media. Bellis 38% WG fungicide was the most effective in decreasing the linear growth diameter of *A. solani* isolates followed by Amistar-top 32.5%SC and Luna experience 40% SC respectively. While, Foliogold 53.75% SC gave the lowest effect. Data in Table (4) showed that, all concentrations of tested fungicides significantly decreased the linear growth of *A. solani* isolates compared with control.

Table 3: Effect of different concentrations of the four chemical inducers on *A. solani* isolates under greenhouse conditions.

Antioxidant	Disease severity (%)						
	Conc. (ppm)	Aggressive			Conc. (ppm)	Non- Aggressive		
		2015	2016	Mean		2015	2016	Mean
Ascorbic acid	3000	38.26	36.9	37.58	3000	10.86	11.3	11.08
Salicylic acid	3000	20.13	18.73	19.43	2000	0	0	0
Citric acid	3000	31.56	28.16	29.86	2500	2.3	3.4	2.85
Catechol	3000	23.23	25.06	24.14	3000	5.86	7.2	6.53
Control		56.0	53.6	54.8	23.16	24.23	23.16	23.69
L.S.D at 0.5%		4.11	4.37			3.39	2.92	

Table 4: Effect of the different concentrations of four fungicides on the linear growth of *A. solani* isolates.

Fungicides	Conc. (ppm)	Mycelial growth inhibition (%)	
		Aggressive	Non-aggressive
Bellis 38%	5	21.3	70.6
	10	47.6	87.3
	25	68.3	100
	50	85.3	100
	100	100	100
	150	100	100
	200	100	100
	250	100	100
	300	100	100
Amistar-top 32.5%	5	15.6	57.3
	10	23.3	60.6
	25	47.3	73.3
	50	55.6	83.3
	100	64.6	96.3
	150	83.3	100
	200	95.6	100
	250	100	100
	300	100	100
Foliogold 53.75%	5	12.6	34.6
	10	15.3	38.3
	25	17.6	43.3
	50	21.3	47.3
	100	26.3	53.3
	150	33.3	56.6
	200	38.3	63.6
	250	48.6	70.3
	300	57.3	78.3
Luna express 40%	5	14.3	42
	10	23	54.3
	25	39.3	66.6
	50	47.3	79.3
	100	58.3	90.3
	150	66.3	100
	200	77.6	100
	250	93.6	100
	300	100	100
Control		0.00	0.00
Treatment A		0.84	1.14
Conc. B		1.09	0.84
A×B		2.44	1.89

3.3.2 *In vivo*

The most effective concentration of each of the four fungicides (Bellis 38% WG, Amistar-top 32.5% SC, Folio gold 53.75% SC and Luna experience 40% SC) were also tested for its effect on disease severity of *A. solani* under greenhouse conditions. Data presented in Table (5) showed that all tested fungicides decreased the disease severity.

Table 5: Effect of certain concentrations of four fungicides on *A. solani* isolates under greenhouse conditions.

Fungicides	Disease severity%							
	Conc. (ppm)	Aggressive			Conc. (ppm)	Non- Aggressive		
		2015	2016	Mean		2015	2016	Mean
Bellis 38% WG	50	8.35	5.66	7	25	0	0	0
Amistar-top 32.5% SC	200	13.76	16.13	14.94	150	0	0	0
Foliogold 53.75% SC	300	32.33	30.1	31.21	200	11.46	9.7	10.58
Luna experience 40% SC	250	22.63	26.1	24.36	150	3.8	6.6	5.2
Control		56.0	53.6	54.8	23.16	24.23	23.16	23.69
L.S.D at .05%		3.05	4.52			2.29	3.34	

Results also showed that disease severity decreased with the increasing of fungicides concentrations. Bellis 38% WG fungicide was the most effective in decreasing disease severity of *A. solani* isolates followed by Amistar-top 32.5% SC and Luna experience 40% SC, respectively. While, Foliogold 53.75% SC was the lowest effective fungicide.

4. Discussion

Eight *A. solani* isolates (Assiut-A3, Minia-M, Assiut-A2, Assiut-A1, Sohag-S, Luxor-L2, Luxor-L1, and Qena-Q) were isolated from five governorates in Egypt. Virulence test indicated that all *A. solani* isolates were pathogenic to tomato plants (Super strain B) causing typical symptoms of the early blight disease. Likewise, data indicated that Assiut-A3 isolate proved to be the most aggressive, recording the highest disease severity percentage, followed by isolates Minia-M, Assiut-A2, respectively. Meanwhile, the other isolates, Assiut-A1, Sohag-S, Luxor-L2, Luxor-L1, and Qena-Q gave the lowest virulent. The previous results indicated that the eight tested isolates were probably different physiological races. Similar results were obtained about *A. solani* isolates and the variation between them (Burm et al., 1995). Differences in the pathogenicity of tested isolates might be due to one or more several factors related to genetic makeup of host variety and pathogen as far as their interaction (Henning et al., 1959). In addition, a high genetic diversity was detected among the *A. solani* isolates (Van der Waals et al., 2004). *Alternaria solani* production of non-specific as well as host specific toxins in the case of pathogenic species (Thomma et al., 2003). Germination fluids of *Alternaria solani* contain alternaric acid as well as a nontoxic substance that acts as susceptibility – inducing factor (Langsdrof et al., 1991). Also, the disease weakens progressively the plant and increases susceptibility to infection by reducing the photosynthetic leaf area and the imbalance between nutrient in the fruits and nutrient supply from the

leaves (Rowell et al., 1953). Statistical analysis revealed significance between linear growth values of the evaluated antioxidants. The lowest linear growth value was achieved by salicylic acid followed by citric acid and catechol, respectively. While, the lowest effective evaluated compound in reducing mycelial growth was ascorbic acid that gave the highest general mean of linear growth. The inhibitory effect of some antioxidants to the growth of *A. solani* were investigated by many researchers (Abada et al., 2008; Abdel-Sayed et al., 2006; Tofali et al., 2003). Several investigators reported that the antioxidants may control seed and soil-borne fungal diseases (Dmitrier et al., 2003; Shahda et al., 2001), as well as foliar fungal diseases (Hassan et al., 2006). Organic acids are known for years for their antibacterial and antifungal properties which have been widely used in foodstuff industry and agriculture (Pao et al., 2008; Sathe et al., 2007; De Muynck et al., 2004). Also, El-Saidy Aml and Abd El-Hai (2011) found that acids effectively controlled fungi. The activation of SAR is associated with the heightened level of expression of the pathogenesis-related proteins, some of which possess antimicrobial activity (Chaturvedi et al., 2007). Salicylic acid (SA) is an important endogenous molecule involved in plant defense. The link between SA production and systemic acquired resistance (SAR) has been well established **(**Delaney et al., 1997; Klessig et al., 1994). Transgenic plants expressing the salicylate dehydrogenase (nahG) gene, which converts SA into inactive catechol, and, do not establish SAR (Gaffney et al., 1993). Furthermore, there is a correlation between the increase in SA levels and plant gene expression. Pathogenesis-related (PR) proteins show up a few hours after the SA level begins to rise (Yalpani et al., 1993). Exogenous SA can induce simultaneous PR expression and resistance to pathogens, even in the absence of pathogenic organisms (Ward et al., 1991). Results also showed that disease severity decreased with the increasing of fungicides concentrations. Bellis 38 % WG fungicide was the most effective fungicide decreasing disease severity and linear growth diameter of *A. solani* isolates followed by Amistar-top 32.5% SC and Luna experience 40% SC respectively. While, Foliogold 53.75% SC was less effective. Similar results were obtained by (Horsfield et al., 2010; Capriotti et al., 2005). Tofoli et al. (2003) who reported that tebuconazole and difenoconazole provided an important and partial inhibition of conidium germination, respectively. In that study, azoxystrobin and pyraclostrobin+methiram showed a moderate inhibitory effect on mycelial growth and complete inhibition of conidium germination starting from 1 mg/ ml, while chlorothalonil and mancozeb demonstrated minor inhibitory levels but superior nevertheless to the control (Pasche et al., 2004). Pyraclostrobin, which significantly reduced the early blight and increased the yield in tomato and potato has reported by many workers (Ganeshan et al., 2009; MacDonald et al., 2007; Ivey et al., 2004). Pyraclostrobin alternated with maneb and pyraclostrobin + boscalid alternated with maneb significantly reduced the anthracnose incidence in bell pepper as compared to control. Best disease management with an improved

yields and fruit quality was reported in combination product of pyraclostrobin+metiram which was effective against both early blight andlate blight has reported by (Capriotti et al., 2005). Horsfield et al. (2010) reported that boscalid was active in the control of early blight disease of potatoes. (Jambhulkar et al., 2012) have reported that spray of azoxystrobin 23% SC showed promising results by reducing disease severity by 38.9% as compared with control. Among the four fungicides analyzed in our present study, Bellis 38% WG and Amistar-top 32.5% SC demonstrated a highest toxicity against *A. solani*. The tested isolates were moderately sensitive to Luna express 40% SC. While, Foliogold 53.75% SC showed low toxicity against isolates.

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