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Control of Tomato Early Blight Disease by Certain Aqueous Plant Extracts

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Abstract: The objective of this work to study the effect of six plant extracts, *Ocimum basilicum* (Sweat Basil), *Azadirachta indica* (Neem), *Eucalyptus chamadulonsis* (Eucalyptus), *Datura stramonium* (Jimsonweed), *Nerium oleander* (Oleander) and *Allium sativum* (Garlic) against *Alternaria solani in vitro* and *in vivo*. In *in vitro* study the leaf extracts of *D. stramonium, A. indica* and *A. sativum* at 5% concentration caused highest reduction of mycelial growth of *A. solani* (44.4, 43.3 and 42.2%, respectively), while *O. basilicum* at 1 and 5% and *N. oleander* at 5% caused the lowest inhibition of mycelia growth of the pathogen. In greenhouse experiments the highest reduction of disease severity was achieved by fungicide (Ridomil Plus 50% WP, 15% metalaxy+35% Copper oxychloride, at 2 g L^{-1}) 82.8% followed by the extracts of *A. sativum* at 5% and *D. stramonium* at 1 and 5% concentration. The greatest reduction of disease severity was achieved by Ridomil Plus 74.2% followed by *A. sativum* at 5% and the smallest reduction was obtained when tomato plant was treated with *O. basilicum* at 1 and 5% (46.1 and 45.2%, respectively). Fungicide, *D. stramonium* and *A. sativum* at 5% increased the fruit yield 85.7, 76.2 and 66.7% compared to infected control. All treatments, plant extracts and fungicide (Ridomil Plus), significantly reduced the early blight disease as well as increased the yield of tomato compared to infected control under field condition.

Key words: *Alternaria solani,* plant extracts, antimicrobial activity, early blight, tomato

INTRODUCTION

Under Egyptian conditions tomato plants are vulnerable to infection early blight disease caused by *Alternaria solani* (Ellis and Martin) Sorauer (Abada *et al*., 2008) which causes great reduction in the quantity and quality of fruit yield. The *Alternaria* fungus can cause disease on all parts of the plant (leaf blight, stem collar rot and fruit lesions) and result in severe damage during all stages of plant development (Abada *et al*., 2008).

This disease is controlled mainly with agro chemicals. However, the world wide trend towards environmentally-safe methods of plant disease control in sustainable agriculture calls for reducing the use of these synthetic chemical fungicides. In an attempt to modify this condition some alternative methods of control have been adopted. Recent efforts have focused on developing environmentally safe, long lasting and effective biocontrol methods for the management of plant diseases. Natural plant products are important sources of new agrochemicals for the control of plant diseases (Kagale *et al*., 2004). Furthermore, biocides of plant origin are non-phytotoxic, systemic and easily biodegradable (Qasem and Aau-Blan, 1996). It is now known that various natural plant products can reduce populations of foliar pathogens and control disease development and then these plant extracts have potential as environmentally safe alternatives and as components in integrated pest management programs (Bowers and Locke, 2004). A number of plant species have been reported to possess natural substances that are toxic to several plant pathogenic fungi (Goussous *et al*., 2010). Dushyent and Bohra (1997) studied the effect of 11 different plant extracts on mycelial growth of *A. solani* and found that leaf extracts of some plants *i.e. Tamarix aphylla* and *Salsola baryosma* totally inhibited the growth of the pathogen *in vivo*. Also, Wszelaki and Miller (2005) reported that garlic extracts significantly reduced the early blight disease on tomato. Additionally, several plant extracts have shown antimicrobial activity against fungal pathogens under *in vitro* and *in vivo* conditions (Kagale *et al*., 2004). Therefore, our present study investigated the efficacy of various Egyptian plants leaf extracts, *Ocimum basilicum, Azadirachta indica, Eucalyptus chamadulonsis, Datura stramonium, Nerium oleander* and *Allium sativum* for control of early blight of tomato under greenhouse and field conditions. The treatments were compared with a commonly used fungicide (Ridomil Plus).

MATERIALS AND METHODS

Plant materials: Seeds of tomato (*Solanum lycopericum* L.) cultivar Super Strain B were obtained from the Ministry of Agriculture, Egypt and used in this study. Seeds were sown in plastic pots, each of 30 cm diameter and

containing a soil mixture consisting of sand 3 kg pot and 10 g slow-release fertilizer per kg (N.P.K 12: 4: 6). All pots were placed on a benchtop in a climate controlled greenhouse at 30±5°C with 68-80% RH and watered as required.

Isolation and pathogenicity tests of the causal pathogen: Six fungal isolates were isolated from naturally diseased tomato leaves and fruits showing blight symptoms. Pathogenicity tests of *Alternaria* sp. isolates were carried out under greenhouse conditions in 2007-2008 experiments in greenhouse of Plant Pathology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt. The inoculum was prepared by growing each of the tested isolates on PDA medium at 27°C for 15 days. Then 10 mL of sterile distilled water was added to each plate and colonies were carefully scraped with a sterile needle. The resulting conidial suspension from each isolate was adjusted to 5×10^6 spores mL^{-1} and used for inoculation of 20 tomato plants (cv. Super Strain B), using an atomizer. After inoculation, plants were covered with polyethylene bags for 48 h to maintain a high humidity conditions. After 48 h, bags were removed and plants were kept under greenhouse conditions. Pots were maintained in completely randomized design under glasshouse conditions. Two weeks after inoculation, disease severity was recorded. The trial was repeated twice. The intensity of disease was recorded in each treatment following the score chart 0-9 scale (0-Healthy; 1 = 1 to 5%; 2 = 6 to 10%; 3 = 11 to 25%; 5 = 26 to 50% and 7 = 51-75% 9 = >76% leaf area infected) proposed by Latha *et al.* (2009).

Preparation of extracts: Extracts from leaves of six plants namely, *O. basilicum, A. indica*, *E. chamadulonsis*, *D. stramonium*, *N. oleander* and *A. sativum* were collected from different parts of Assiut, Egypt and tested for their efficacy in reducing the mycelial growth of *A. solani in vitro* using the poisoned food technique (Schmitz, 1930). Ten grams of fresh leaf material of each plant species was collected, washed with water and crushed in a mortar and pestle by adding sterile distilled water at the rate of 10 mL g^{-1} of plant tissue and the homogenates were centrifuged at 10000xg for 15 min at 4°C and the supernatant solutions were collected. The plant extract was diluted further to have 1 and 5% concentration (v/v). These fractions were sterilized using 0.2 m disposable syringe filters and used for assay of antimicrobial activity as described below.

The PDA media amended with five milliliters of aqueous leaf extract, 1 and 5%, of each plant extracts individually were inoculated with mycelial discs (9 mm diameter) taken from the advancing edges of 7 day-old pure cultures of *A. solani*. The control experiments had distilled water instead of plant extracts. The inoculated media were incubated at temperature 27±1°C. Four plates were each treatment was used as a replicates. The diameter of the fungal colony was measured using a meter rule along two diagonal lines drawn on the reverse side of each Petri plate 7 days after inoculation. Each treatment was replicated three times with four plates per replication.

Testing of plant extracts against early blight of tomato under greenhouse conditions: Fungicide (Ridomil Plus 50% WP, 15% metalaxyl+35% Copper oxychloride, at 2 g L^{-1}) and plant extracts treatments at 1 and 5% were applied as foliar application, 30 mL on tomato plants, seven week olds and every 15 days up to 60 days of planting after two days from second spraying tomato plants were inoculated with 20 mL of *A. solani* suspension containing 5×10^6 cfu mL^{-1}. After inoculation, plants were kept in a climate chamber with 28°C day temperature and 85% relative humidity. Disease development was recorded 15 days after inoculation. Disease severity was recorded as described before. Greenhouse experiments were repeated twice.

Testing of plant extracts on early blight of tomato under field conditions: The field trials were conducted at the Experimental Farm of Faculty of Agriculture, Assiut University, Assiut, Egypt in 2008 and 2009 growing seasons. Field plots (3×3.5 m) comprised two rows and 5 plants/row arranged in a completely randomized block design. Three plots were used as replicates for each treatment as well as for the untreated control treatment. Application of plant extracts was carried out as in greenhouse experiments. Disease development was recorded 15 days after inoculation. Disease severity was recorded as described before. Field experiments were repeated twice. At harvest time, the average accumulated yield was calculated for each treatments including untreated control. Ten plants from each replicate were harvested to assess the total yield of each treatment (ton ha^{-1}).

Statistical analysis: All experiments were performed twice. Analyses showed no significant interaction between the two tests run for any of the treatment. Therefore, results from duplicate tests were combined for final analysis. Analyses of variance were carried out using MSTAT-C program version 2.10 (MSTAT-C 1991). Least

Significant Difference (LSD) was employed to test for significant difference between treatments at p = 0.05 (Gomez and Gomez, 1984).

RESULTS

Identification of the causal pathogen: Six fungal isolates were obtained from naturally diseased tomato leaves and fruits showing blight symptoms and identified as *A. solani*, based on the morphological characteristics (Ellis, 1976).

Pathogenicity tests: Results in Fig. 1 indicate that all the tested isolates of *A. solani* were able to infect tomato plants causing typical early blight symptoms with different degrees of disease severity. Data indicate that isolates 1, 3 and 5 were highly pathogenic and caused the highest disease severity. Isolates 2 and 4 exhibited the lowest disease severity on tomato plants followed by isolate 6. On the basis of this result, isolate 1 was used in the following experiments.

Effect of plant extracts on radial growth of *A. solani*: Six plant species were selected and evaluated for the antimicrobial activity against *A. solani*. All the leaf extracts of tested plants at 1 and 5% concentration were effective in inhibiting the radial growth of *A. solani*, compared to control. The leaf extract of *D. stramonium*, *A. indica* and *A. sativum* at 5% concentration caused highest reduction of mycelial growth of *A. solani* (44.4, 43.3 and 42.2%, respectively), followed by *E. chamadulonsis* and *D. stramonium* at 1% concentration. *O. basilicium* at 1 and 5% and *N. oleander* at 1% caused the lowest inhibition of mycelial growth of the pathogen. Overall the Ridomil Plus at 2 g L^{-1} caused the highest reduction of the pathogen by 77.8% (Table 1).

Effect of plant extracts on early blight incidence of tomato under artificial infection in greenhouse conditions: The different concentrations of six plant extracts, *O. basilicum*, *A. indica*, *E. chamadulonsis*, *D. stramonium*, *N. oleander* and *A. sativum*, significantly reduced the early blight diseases (Table 2). The greatest reduction 82.8% of disease severity was achieved by Ridomil Plus at 2 g L^{-1}. The most effective treatments from plant extracts were *A. sativum* at 1 and 5% followed by *D. stramonium* at 1 and 5% concentration. The least reduction of disease severity was achieved by *O. basilicum* at 1% (35.2%). Other plant extracts treatments were moderately effective.

Effect of some plant extracts on early blight incidence of tomato under field conditions: All treatments, plant extracts and fungicide (Ridomil Plus at 2 g L^{-1}),

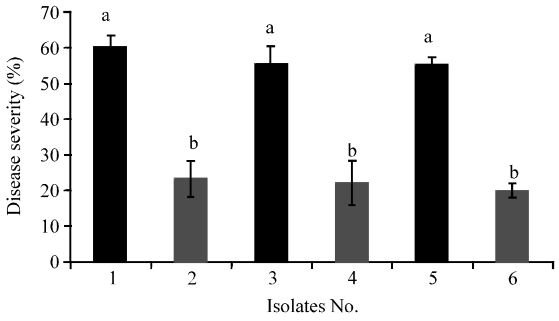

Fig. 1: Pathogenicity tests of six isolates of *Alternaria solani* on tomato plants (cv. Super Strain B) under greenhouse conditions. Different letters indicate significant differences among treatments according to least significant difference test (p = 0.05). Means of standard deviation for twenty plants per treatment are shown

Table 1: *In vitro* effect of six plants extracts on the linear growth of *Alternaria solani*

Treatments	Concentration [a]	Linear growth (cm) [b]	Percent reduction
O. basilicum	1	7.0^{b}	22.2
	5	6.9^{b}	23.3
A. indica	1	6.2^{c}	31.1
	5	5.1^{d}	43.3
E. chamadulonsis	1	6.5^{c}	27.8
	5	6.3^{c}	30.0
D. stramonium	1	5.5^{d}	38.9
	5	5.0^{d}	44.4
Nerium oleander	1	6.9^{b}	23.3
	5	6.1^{c}	32.2
A. Sativum	1	6.1^{c}	32.2
	5	5.2^{d}	42.2
Ridomil Plus g L^{-1}	2	2.0^{e}	77.8
Control		9.0^{a}	0.0

A five milliliters of aqueous leaf extracts prepared from each of the plant sample was mixed with 45 mL of PDA medium (1 and 5%). [b] The percent inhibition of radial growth of *A. solani* was calculated. Each treatment was replicated three times with four plates per replication. Values in the column followed by the same letter are not significantly different at (p = 0.05)

Table 2: Influence of six plants extracts on early blight disease of tomato plants under greenhouse conditions

Treatments	Concentration (%)	Disease severity (%)	Percent reduction
O. basilicum	1	35.2^{b}	34.9
	5	30.3^{c}	44.0
A. indica	1	27.8^{c}	48.6
	5	24.3^{cd}	55.1
E. chamadulonsis	1	28.7^{c}	47.0
	5	26.4^{c}	51.2
D. stramonium	1	19.4^{de}	64.1
	5	17.2^{de}	68.2
Nerium oleander	1	30.0^{c}	44.5
	5	25.9^{c}	52.1
A. Sativum	1	20.8^{cd}	61.6
	5	15.3^{f}	71.7
Ridomil plus g L^{-1}	2	9.3^{g}	82.8
Infected Control		54.1^{a}	0.0

The intensity of the disease was recorded in each treatment as proposed by Latha *et al.* (2009). Values in the column followed by different letters indicate significant differences among treatments according to least significant difference test (p = 0.05)

Table 3: Influence of six plant extracts on early blight disease and yield of tomato under field conditions

Treatments	Concentration (%)	Disease severity (%)	Percent reduction	Yield (t ha^{-1})	Percent increase (%)
O. basilicum	1	46.1^{b}	22.1	2.7^{cb}	28.6
	5	45.2^{b}	23.6	2.8^{cb}	33.3
A. indica	1	41.3^{c}	30.2	2.8^{cb}	33.3
	5	38.2^{d}	35.5	2.8^{cb}	33.3
E. chamadulonsis	1	37.9^{d}	36.0	2.8^{cb}	33.3
	5	36.2^{d}	38.9	3.1^{b}	47.6
D. stramonium	1	28.4^{f}	52.0	3.1^{b}	47.6
	5	27.1^{f}	54.2	3.7^{a}	76.2
Nerium oleander	1	37.2^{d}	37.2	2.9^{cb}	38.1
	5	35.1^{e}	40.7	3.0^{b}	42.9
A. sativum	1	27.3^{f}	53.9	3.2^{b}	52.4
	5	25.1^{g}	57.6	3.5^{a}	66.7
Ridomil plus g L^{-1}	2	15.3^{h}	74.2	3.9^{a}	85.7
Infected control		59.2^{a}	0.0	2.1^{d}	0.0

Values in the column followed by different letters indicate significant differences among treatments according to least significant difference test (p = 0.05)

significantly reduced the early blight disease under field conditions (Table 3). The greatest reduction of disease severity at 74.2% was achieved by Ridomil Plus at 2 g L^{-1} followed by *A. sativum* at 5% and the least reduction was obtained when tomato plant were treated with *O. basilicum* at 1 and 5% (46.1 and 45.2 %, respectively). The other treatments were moderately effective.

Effect of treatments on fruit yield: Data in Table 3 indicate that the efficacy of the Ridomil Plus and plant extracts was reflected in the fruit yield produced. Plants sprayed with fungicide, *D. stramonium* and *A. sativum* at 5% increased the fruit yield 85.7, 76.2 and 66.7% respectively, compared to nontreated control. In contrast, *O. basilicum, A. indica*, *E. chamadulonsis* and *N. oleander* treatments increased the fruit yield moderately, ranged between 28.6 to 38.1% compared to infected control.

DISCUSSION

Our results indicated that all tested plant extracts, *Ocimum basilicum, Azadirachta indica*, *Eucalyptus chamadulonsis*, *Datura stramonium*, *Nerium oleander* and *Allium sativum* and fungicide, Ridomil Plus 50% WP, caused significant reduction in the linear growth of *A. solani*. This reduction was gradually increased by increasing the concentration of extracts in the growth medium. Ridomil Plus was more effective than the plant extracts. Similar effect of various other plant products effective against *Alternaria* spp. have been reported by several workers (Latha *et al*., 2009; Goussous *et al*., 2010). Vijayan (1989) reported that the bulb extract of *A. sativum*, leaf extract of *Aegle marmelos* and flower extract of *Catharanthus roseus* inhibited the spore germination and mycelial growth of *A. solani*. The inhibitory effect of the fungicide on the growth of *A. solani* was reported by many researchers (Patil *et al*., 2001; Abada *et al*., 2008). The inhibitory effect of the tested plant extracts may be due to their direct toxic effect to the pathogen as reported by Vijayan (1989). Investigations on mechanisms of disease suppression by plant products have suggested that the active principles present in plant extracts may either act on the pathogen directly (Amadioha, 2000), or induce systemic resistance in host plants resulting in reduction of disease development (Kagale *et al*., 2004).

The greenhouse and field experiments indicated that foliar spray of tomato plants by plant extracts and fungicide resulted in significant reduction in early blight infection. However, the tested fungicide was more efficient than plant extracts. These results were similar to previous work on the role of plant extracts in fungal disease control. Several authors including Krebs *et al*. (2006), Curtis *et al*. (2004) and Latha *et al*. (2009) reported that plant extracts from 20 non-host plant species caused reduction of early blight disease and suppressed the mycelial growth of *A. solani*. All tested plant extracts treatments improved the yield of tomato plants compared to infected control.

In conclusion, present study demonstrated that many plant extracts, *O. basilicum,* A. indica, *E. chamadulonsis*, *D. stramonium*, *N. oleander* and *A. sativum,* can be used for the bio-control of early blight disease. Thus, this method of control can contribute to minimizing the risks and hazards of toxic fungicides, especially on vegetables produced for fresh consumption. Further research into these extracts will identify the active compounds responsible for their fungicidal activity.

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