Evaluation of Various Plant Extracts against the Early Blight Disease of Tomato Plants under Greenhouse and Field Conditions

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Abstract

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The antimicrobial activity of six plant extracts from *Ocimum basilicum* (Sweat Basil), *Azadirachta indica* (Neem), *Eucalyptus chamadulonsis* (Eucalyptus), *Datura stramonium* (Jimsonweed), *Nerium oleander* (Oleander), and *Allium sativum* (Garlic) was tested for controlling *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 the highest reduction of mycelial growth of *A. solani* (44.4, 43.3 and 42.2%, respectively), while *O. basilicum* at 1% and 5% concentration and *N. oleander* at 5% concentration caused the lowest inhibition of mycelial growth of the pathogen. In greenhouse experiments the highest reduction of disease severity was achieved by the extracts of *A. sativum* at 5% concentration and *D. stramonium* at 1% and 5% concentration. The greatest reduction of disease severity was achieved by *A. sativum* at 5% concentration and the smallest reduction was obtained when tomato plants were treated with *O. basilicum* at 1% and 5% concentration (46.1 and 45.2 %, respectively). *D. stramonium* and *A. sativum* at 5% concentration increased the fruit yield by 76.2% and 66.7% compared to the infected control. All treatments with plant extracts significantly reduced the early blight disease as well as increased the yield of tomato compared to the infected control under field conditions.

Keywords: *Alternaria solani*; Neem; Sweat Basil; Eucalyptus; Oleander; Garlic; Jimsonweed; antimicrobial activity

Under Egyptian conditions tomato plants are vulnerable to infection with the early blight disease caused by *Alternaria solani* (Ellis & Martin) Sorauer (Abada *et al.* 2008), which causes a great reduction in the quantity and quality of fruit yield. The *Alternaria* fungus can cause the 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 by the application of agrochemicals. However, the worldwide 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 the 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 & Abu-Blan1966). It is now known that various natural plant products can reduce populations of foliar pathogens and control the disease development, and then these plant extracts have a potential as environmentally safe alternatives and as components in integrated pest management programs (Bowers & 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 the 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*. Wszelaki and Miller (2005) also reported that garlic extracts significantly reduced the early blight disease on tomato. Additionally, several plant extracts have shown the 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 plant leaf extracts from *Ocimum basilicum*, *Azadirachta indica*, *Eucalyptus chamadulonsis*, *Datura stramonium*, *Nerium oleander*, and *Allium sativum* in the control of early blight of tomato under greenhouse and field conditions.

MATERIAL AND METHODS

Plant materials. Seeds of the tomato (*Solanum lycopericum* L.) cultivar Super Strain B were obtained from the Ministry of Agriculture, Egypt. Seeds were sown into plastic pots, each of 30 cm diameter and containing a soil mixture consisting of sand 3 kg/pot and 10 g slow-release fertiliser 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 infected tomato leaves and fruits showing blight symptoms. Pathogenicity tests of *Alternaria* sp. isolates were carried out under greenhouse conditions in 2008–2009 experiments in a greenhouse of Plant Pathology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt. The inoculum was prepared by culturing 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 and used for the inoculation of 20 tomato plants (cv. Super Strain B) using an atomiser. After inoculation, plants were covered with polyethylene bags for 48 h to maintain high humidity conditions. After 48 h, bags were removed and plants were kept under greenhouse conditions. Pots were arranged in a completely randomised design under greenhouse conditions. Two weeks after inoculation, disease severity was recorded. The experiment was repeated twice. The intensity of disease was recorded in each treatment following the score chart 0–9 scale (0 = healthy; 1 = 1–5%; 2 = 6–10%; 3 = 11–25%; 5 = 6–50%, 7 = 51–75%, and 9 = > 76% of the 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 the fresh leaf material of each plant species were collected, washed with water and crushed in a mortar with pestle by adding sterile distilled water at the rate of 10 ml/g of plant tissue and the homogenates were centrifuged at 10 000× *g* for 15 min at 4°C and the supernatant solutions were collected. The plant extract was further diluted to have 1% and 5% concentration (v/v). These fractions were sterilised with 0.2 m disposable syringe filters and used for assays of antimicrobial activity as described below.

The PDA media amended with 5 ml of aqueous leaf extract, 1% and 5% of each plant extract individually, were inoculated with mycelial discs (9 mm in diameter) taken from the advancing edges of 7-day-old pure cultures of *A. solani.* Distilled water instead of plant extracts was used in the control experiments. The inoculated media were incubated at a temperature of 27 ± 1°C. Four plates per each treatment were used as replications. 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. Treatments with plant extracts at 1% and 5% concentrations were performed as foliar application, 30 ml to tomato plants, seven weeks old, and every 15 days up to 60 days after planting; after two days from the second spraying tomato plants were inoculated with 20 ml of *A. solani* suspension containing 5×10^6 CFU/ml. After inoculation, plants were kept in a climate chamber at a daily temperature of 28°C and 85% relative humidity. Disease devel-

opment was recorded 15 days after inoculation. Disease severity was recorded as described before. Greenhouse experiments were repeated twice.

Testing of plant extracts for 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 2009 and 2010 growing seasons. Field plots (3 × 3.5 m) comprised two rows and 5 plants per row arranged in a completely randomised block design. Three plots were used as replications for each treatment as well as for the untreated control treatment. The application of plant extracts was carried out like in greenhouse experiments. Disease development was recorded 15 days after inoculation. Disease severity was recorded as described before. Field trials were repeated twice. At harvest time, the average accumulated yield was calculated for each treatment including the untreated control. Ten plants from each replication were harvested to assess the total yield of each treatment (t/ha)

Statistical analysis. All experiments were performed twice. Analyses showed no significant interaction between the two tests run for any of the treatments. Therefore, results from duplicate tests were combined for the final analysis. Analyses of variance were carried out using MSTAT-C program version 2.10 (1991). The least significant difference (*LSD*) was employed to test for significant differences between treatments at $P \leq 0.05$ (Gomez & Gomez 1984).

RESULTS

Identification of causal pathogen

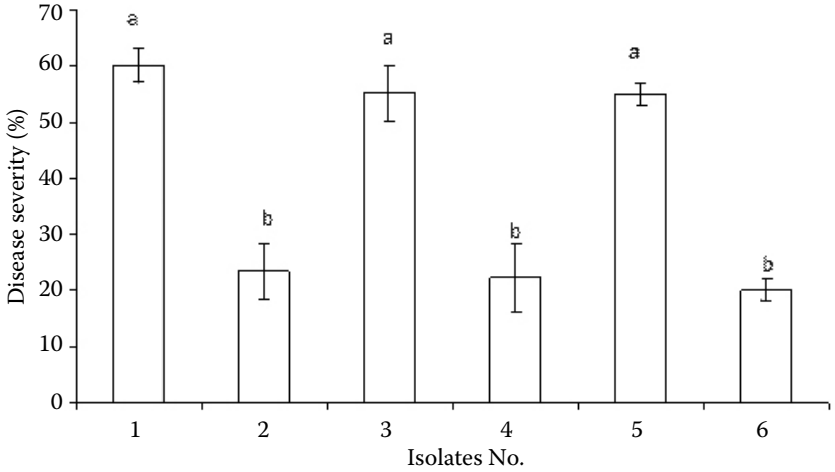
Six fungal isolates were obtained from naturally infected tomato leaves and fruits showing blight symptoms and identified as *A. solani*, based on the morphological characteristics (Ellis 1976).

Pathogenicity tests

Results in Figure 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 document 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 No. 1 was used in subsequent 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 the control.



Different letters indicate significant differences among treatments according to the least significant difference test (P = 0.05); means of standard deviations for twenty plants per treatment are shown

Figure 1. Pathogenicity tests of six isolates of *Alternaria solani* on tomato plants (cv. Super Strain B) under greenhouse conditions

Table 1. *In vitro* effects of six plant extracts on the linear growth of *Alternaria solani*

Treatments	Concentration (%)*	Linear growth (cm)**	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
N. 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
Control		9.0[a]	0.0

*F5 mm of aqueous leaf extracts prepared from each of the plant sample was mixed with 45 ml of PDA medium (1% and 5% concentration); **percent inhibition of the 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 according to the *LSD* test at 0.05

The leaf extract of *D. stramonium, A. indica*, and *A. sativum* at 5% concentration caused the highest reduction of the 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% concentration and *N. oleander* at 1% concentration caused the lowest inhibition of the mycelial growth of the pathogen (Table 1).

Effect of plant extracts on early blight incidence in 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 disease (Table 2). The most effective treatments with plant extracts were *A. sativum* at 1% and 5% concentration, followed by *D. stramonium* at 1% and 5% concentration. The least reduction of disease index was achieved by *O. basilicum* at 1% concentration (35.2%). Other treatments with plant extracts were moderately effective.

Effect of some plant extracts on early blight incidence in tomato under field conditions

All treatments with plant extracts significantly reduced the early blight disease under field conditions (Table 2). The greatest reduction of disease severity was achieved by *A. sativum* at 5% concentration and the least reduction was obtained when tomato plants were treated with *O. basilicum* at 1% and 5% concentrations (46.1% and 45.2 %, respectively). The other treatments were moderately effective.

Effect of treatments on fruit yield

Data in Table 2 indicate that the efficacy of plant extracts was reflected in the fruit yield produced. Plants sprayed with *D. stramonium* and *A. sativum* at 5% concentration increased the fruit yield by 76.2% and 66.7%, respectively, compared to the nontreated control. In contrast, *O. basilicum, A. indica*, *E. chamadulonsis* and *N. oleander* treatments increased the fruit yield moderately, in the range between 28.6% and 38.1% compared to the infected control.

Table 2. Influence of six plant extracts on the early blight disease of tomato plants under greenhouse and on the early blight disease and yield of tomato under field conditions

Treatments	Concentration (%)	Greenhouse conditions		Field conditions			
		disease severity (%)	reduction (%)	disease severity (%)	reduction (%)	yield (t/ha)	increase (%)
O. basilicum	1	35.2^{b}	34.9	46.1^{b}	22.1	2.7^{cb}	28.6
	5	30.3^{c}	44.0	45.2^{b}	23.6	2.8^{cb}	33.3
A. indica	1	27.8^{c}	48.6	41.3^{c}	30.2	2.8^{cb}	33.3
	5	24.3^{cd}	55.1	38.2^{d}	35.5	2.8^{cb}	33.3
E. chamadulonsis	1	28.7c	47.0	37.9^{d}	36.0	2.8^{cb}	33.3
	5	26.4^{c}	51.2	36.2^{d}	38.9	3.1^{b}	47.6
D. stramonium	1	19.4^{de}	64.1	28.4^{f}	52.0	3.1^{b}	47.6
	5	17.2^{de}	68.2	27.1^{f}	54.2	3.7^{a}	76.2
N. oleander	1	30.0^{c}	44.5	37.2^{d}	37.2	2.9^{cb}	38.1
	5	25.9^{c}	52.1	35.1^{e}	40.7	3.0^{b}	42.9
A. sativum	1	20.8^{cd}	61.6	27.3^{f}	53.9	3.2^{b}	52.4
	5	15.3^{f}	71.7	25.1^{g}	57.6	3.5^{a}	66.7
Infected control		54.1^{a}	0.0	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 the least significant difference test (P = 0.05)

DISCUSSION

Our results indicated that all tested plant extracts, *Ocimum basilicum*, *Azadirachta indica*, *Eucalyptus chamadulonsis*, *Datura stramonium*, *Nerium oleander*, and *Allium sativum*, caused a significant reduction in the linear growth of *A. solani*. This reduction was gradually increased by increasing the concentration of extracts in the growth medium. Similar effects of various other plant products effective against *Alternaria* spp. were reported by several authors (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 tested plant extracts may be due to their direct toxic effect on the pathogen as reported by Vijayan (1989). Investigations on the 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 a reduction of the disease development (Kagale *et al.* 2004).

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

In conclusion, our study demonstrated that many plant extracts, e.g. from *O. basilicum*, *A. indica*, *E. chamadulonsis*, *D. stramonium*, *N. oleander*, and *A. sativum,* can be used for the biocontrol of the early blight disease. Thus, this method of control can contribute to minimising 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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