

Full Length Research Paper

***In vitro* antifungal evaluation of various plant extracts against early blight disease (*Alternaria solani*) of potato**

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Accepted 23 May, 2011

Antifungal activities of 27 plant extracts were tested against *Alternaria solani* (E. & M.) Jones and Grout using radial growth technique. While all tested plant extracts produced some antifungal activities, the results revealed that *Cirsium arvense*, *Humulus lupulus*, *Lauris nobilis* and *Salvia officinalis* showed significant antifungal activities. The leaf extract of *L. nobilis* was most effective in inhibiting the mycelial growth of *A. solani* (79.35%) at 4% concentration, followed by *S. officinalis*, *H. lupulus*, and *C. arvense* with 76.50, 61.50 and 55.83% inhibition, respectively. The other tested plant extracts exhibited moderate activity and average mycelial growth inhibition of fungus varied from 9.15 to 50.58%. The lowest antifungal activity was observed on *Hypericum perforatum* extract. The antifungal activity of extracts of *C. arvense*, *H. lupulus*, *L. nobilis* and *S. officinalis* were further evaluated at different concentrations (0.2, 0.4, 2, 4 and 8% (w/v)) against *A. solani*. Inhibitory effects of these extracts were increased with increasing concentration. The minimum inhibitory concentration (MIC) of *H. lupulus*, *L. nobilis* and *S. officinalis* were 8% (w/v). Further studies on isolation and characterization of the active (antifungal) compound is needed before the possible use of the tested extracts in control strategies of this fungus.

Key words: Plant extracts, *Alternaria solani*, antifungal, minimum inhibitory concentration (MIC).

INTRODUCTION

Potato is one of the most important crop in the world and is planted in 18.2 million ha and total yield reached 314.1 million ton (FAO, 2010). In Turkey, it is grown in an area of 154000 ha with an annual production of 4.3 million ton (FAO, 2010). Potato plants are subjected to attack by numerous diseases wherever the crop is planted. Fungal pathogens, *Alternaria solani* (E. & M.) Jones and Grout causes early blight disease of potato and occurs annually to some degree in most production areas. The timing of appearance and rate of disease progression help to determine the impact of the disease on the potato crop. The disease occurs over a wide range of climatic conditions and depends in a large part, on the frequency of foliage wetting from rainfall, fog, dew, or irrigation, and on the nutritional status of foliage as well as cultivar

susceptibility. It has been reported that severe epidemics can reduce yields by up to 30% (Christ and Maczuga, 1989; Shtienberg et al., 1990). Control of early blight disease has been accomplished primarily by the application of chemical fungicides (Jones et al., 1991).

Several effective pesticides have been recommended for use against this pathogen, but they are not considered to be long-term solutions, due to concerns of expense, exposure risks, fungicide residues and other health and environmental hazards. In an attempt to modify this condition, some alternative methods of control have been adopted. Natural products isolated from plant appear to be one of the alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999). Control of microorganism linked plant disease with plant extracts as components in integrated pest management strategy has been tested by many researchers. Chapagain et al. (2007) reported that saponin rich-extracts (4%) from *Balanites aegyptiaca* fruit mesocarp,

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showed 34.7% growth inhibition against *A. solani*. Also Muto et al. (2005) tested the extracts derived from fresh and dry tissues of 14 plant species against *A. solani*. Mohana and Raveesha (2007) reported that the aqueous extract from *Decalepis hamiltonii* at 30% concentration caused 84.83% mycelial growth inhibition on *A. alternata* and increase in extract concentration up to 50% resulted in 100% inhibition. Report also showed that all tested plant extracts lowered the disease severity of early blight in summer growing season, especially the extracts of lemon grass leaves, garlic bulbs, basil leaves and marjoram leaves, respectively.

The objectives of the present study is to evaluate the antifungal activity of methanolic extracts of 27 plant species against *A. solani* under *in vitro* conditions.

MATERIALS AND METHODS

The pathogenic isolates of *A. solani* (E. & M.) Jones and Grout (AS-5) was isolated from the potato leaves showing typical symptoms of early blight by using potato dextrose agar (PDA) medium and identified as *A. solani* according to Simms (2007).

Plant materials and extract preparation

The extract of twenty seven naturally growing plant species (Table 1) were used in the present study. The plants were collected during spring and summers of 2002 to 2003 from different localities of Taşlıçiftlik, Tokat, a temperate region of Turkey, where the altitude is 640 m and the soil is sandy lime soil, except fruits of *Styrax officinalis* and *Hedera helix* which were collected from Mersin. The plant parts (leaves and fruits) were air dried at room temperature for three weeks in dark conditions. The dried plant parts were milled to a fine powder in a mill (Model M 20 IKA Universal Mill, IKA Group), and stored at room temperature in closed 2000 ml glass jars in the dark, at 20°C until used. Fifty grams each of the dried, powdered plant sample were weighed and placed into 1000 ml Erlenmeyers flasks and then 500 ml of absolute methanol (Sigma-Aldrich) was added to the flask. The flasks were closed with cotton balls and covered with aluminium foil then placed on a horizontal shaker (HS 260 Basic, IKA Group) and shaken at 120 rpm for 24 h in the dark and then the suspension were filtered through two layers of cheese cloth into different 250 ml evaporating flasks. Excess alcohol was evaporated to dryness using a rotary evaporator (RV 05 Basic 1B, IKA Group) at 32 ± 2°C and the remaining residue was diluted by adding appropriate quantity of sterilized distilled water containing 10% acetone (v/v) to prepare 40% (w/v) stock suspension (Gokce et al., 2006). These stock suspensions were stored at 4°C and used within four days.

Screening of plant extracts against *A. solani*

The antifungal properties of the extracts were tested using the radial growth method as described by Bansa et al. (1999). PDA medium was prepared by autoclaving at 121°C and cooled to 45°C. Afterwards, appropriate quantities of stock solution of each extract was added to PDA medium to get 4% (w/v) concentrations of the extracts in the medium and mixed. In the control, 10% acetone (v/v) water mixture was added to PDA. Twenty milliliters of each medium was poured into 90 mm diameter sterilized Petri plates and left to solidify over night. Mycelial discs of 5 mm diameter were taken from 7 days old *A. solani* cultures with a sterilized cork borer and were

placed in the centre of each Petri plate. The position of the disc was marked on the base of the dish with a marker pen and two orthogonal axes passing through the centre of the disc were marked to be used as references for recording growth. Plates were incubated at temperature of 28 ± 2°C for 7 days. Radial growth along each line was recorded at exactly 24 h intervals using callipers (Mitutoyo). The inhibitory activity of each treatment was expressed as the percent growth inhibition as compared to the negative control (0%) using the following formula (Pandey et al., 1982):

$$\text{Growth inhibition (\%)} = \frac{DC - DT}{DC} \times 100$$

Where, DC = Diameter of control and DT = diameter of fungal colony with treatment. Each treatment was replicated four times with five plates per replication.

Determination of minimum inhibitory concentration (MIC)

Based on effects of plant extracts on radial growth experiment, *Cirsium arvense*, *Humulus lupulus*, *Lauris nobilis* and *Salvia officinalis* extracts were further tested for the determination of MIC. Various concentrations (0.2, 0.4, 2, 4 and 8% (w/v)) of the extracts of plant species were prepared by adding appropriate quantities of stock solution of each extract and distilled water to PDA medium and thoroughly mixed with the medium. Twenty milliliters of each medium was poured into each 90 mm diameter sterilized Petri plates. Plates were inoculated, incubated and evaluated as described earlier. Each treatment was replicated four times with five plates per replication. Antracol WP 70 (Propineb %70) (Bayer) was used as a standard, synthetic fungicide for comparison of results under identical conditions. The MIC value was defined as the lowest extract concentration required for complete suppression of mycelial growth of the tested fungus (Barbour et al., 2004).

Statistical analysis

The data on effect of the treatments on the growth of pathogens was analyzed by analysis of variance (ANOVA), and treatment means were compared by Fishers least significant difference test (LSD) at P = 0.05.

RESULTS

Effect of plant extracts on radial growth of *A. solani*

Twenty seven plant species, belonging to the various families were selected and evaluated for antifungal activity. Twenty two out of twenty seven plant extracts at 4% concentration were effective in inhibiting the radial growth of *A. solani* (E. & M.) Jones and Grout isolate (AS-5). The leaf extract of *L. nobilis* was most effective in inhibiting the mycelial growth of *A. solani* (79.35%) at 4% concentration, followed by *S. officinalis*, *H. lupulus* and *C. arvense* with 76.50, 61.50 and 55.83% inhibition, respectively (Table 1). Inhibitory activities of *L. nobilis* and *S. officinalis* extracts were significantly different when compared with the rest of the tested plant extracts. Not much difference was observed in the activities of *H. lupulus* and *C. arvense* extracts in the period of test. On the other hand, activities in the extracts of *E. elaterium*, *H.*

Table 1. Effect of plant extracts on the mycelial growth of *A. solani*.

Family	Plant species	Plant part	Per cent reduction*
Lauraceae	<i>Laurus nobilis</i> L.	Leaves	79.35a
Lamiaceae	<i>Salvia officinalis</i> L.	Leaves	76.50a
Cannabinaceae	<i>Humulus lupulus</i> L.	Flower bud	61.50b
Asteraceae	<i>Cirsium arvense</i> (L.) Scop.	Leaves	55.83bc
Styracaceae	<i>Styrax officinalis</i> L.	Fruit	50.58cd
Apocynaceae	<i>Nerium oleander</i> L.	Leaf	49.75cd
Rubiaceae	<i>Galium aperina</i> L.	Leaves	44.10de
Solanaceae	<i>Solanum nigrum</i> L.	Fruit	40.88e
Chenopodiaceae	<i>Chenopodium album</i> L.	Leaves	36.80e
Caprifoliaceae	<i>Sambucus nigra</i> L.	Fruit	27.90f
Poaceae	<i>Sorghum halepense</i> (L.) Pers.	Fruit	27.03f
Asteraceae	<i>Arctium lapa</i> L.	Leaves	26.28f
Araliaceae	<i>Hedera helix</i> L.	Leaves	25.95f
Fabaceae	<i>Glycyrrhiza glabra</i> L.	Fruit	22.30fg
Solanaceae	<i>Datura stramonium</i> L.	Fruit	22.20fg
Asteraceae	<i>Artemisia vulgaris</i> L.	Leaves	21.38 fg
Poaceae	<i>Cynodon dactylon</i> L.	Leaves	16.63gh
Scrophulariaceae	<i>Verbascum songaricum</i> L.	Leaves	15.20gh
Poaceae	<i>Lolium temulentum</i> L.	Leaves	12.10hi
Asteraceae	<i>Xanthium strumarium</i> L.	Fruit	11.53hi
Urticaceae	<i>Urtica urens</i> L.	Leaves	9.85hi
Rubiaceae	<i>Rubia tinctoria</i> L.	Leaves	9.15hi
Cucurbitaceae	<i>Ecballium elaterium</i> (L.) A. Rich.	Fruit	5.60ij
Guttiferae	<i>Hypericum perforatum</i> L.	Flowers	4.50ij
Ranunculaceae	<i>Delphinium consolida</i> L.	Leaves	0.10j
Asteraceae	<i>Chrysanthemum segetum</i> L.	Leaves	0.00j
Apiaceae	<i>Conium maculatum</i> L.	Leaves	0.00j
	Control (PDA with 10% acetone)		0.00j

*Percentage (%) growth inhibition was calculated with comparison with the growth of the control. In column, means followed by the same letter are not significantly different at 5% level by LSD (7.90).

perforatum and *Delphinium consolida* was not significant, whereas extracts of *Chrysanthemum segetum* and

Conium maculatum did not show any inhibitory activity against *A. solani* (Table 1).

Table 2. Minimum inhibitory concentration of four plant extracts against *A. solani*.

Extract Concentration (% w/v)	Growth inhibition (%)
<i>C. arvense</i> (LSD=6.7)	
0.2	35.8a
0.4	42.9b
2.0	46.2b
4.0	55.8c
8.0	76.8d
<i>L. nobilis</i> (LSD=11.0)	
0.2	24.7a
0.4	26.0a
2.0	58.6b
4.0	79.4c
8.0	100.0d
<i>S. officinalis</i> (LSD=1.6)	
0.2	63.8a
0.4	65.9b
2.0	69.6c
4.0	76.5d
8.0	100.0e
<i>H. lupulus</i> (LSD=5.9)	
0.2	42.4a
0.4	45.0a
2.0	51.5b
4.0	61.5c
8.0	100.0d
Propineb 70% (Antracol WP 70)	100.0
Control (10% acetone)	0.0

*Means followed by same letter in each column are not significantly different at $p = 0.05$ by Fisher's least significant difference value. Percentage (%) growth inhibition was calculated with comparison with the growth of the control (0%).

Determination of MIC

Based on effects of plant extracts on radial growth experiment, *C. arvense*, *H. lupulus*, *L. nobilis* and *S. officinalis* extracts were further tested for determination of MIC. Various concentrations (0.2, 0.4, 2, 4 and 8% (w/v)) of these plants extracts were tested against *A. solani*. Results indicate that inhibition values of the extracts from *C. arvense*, *L. nobilis*, *S. officinalis* leaves and *H. lupulus* flower bud were higher in comparison to the control, and this effect gradually increased with concentration (Table 2). The *C. arvense* leaf extract inhibited mycelial growth of *A. solani* at 35.8% and even at 0.2% (w/v), reaching 76.8% at 8% concentration. On the other hand, *L. nobilis*, *S. officinalis* leaves extracts and *H. Lupulus* flower bud

extract produced similar inhibition at high concentration. Inhibition reached 100% at 8% concentration (w/v) for *L. nobilis*, *S. officinalis* and *H. lupulus*, but for *C. Arvense*, it was higher than 8% (Table 2). The MIC of *L. nobilis*, *S. officinalis* and *H. lupulus* was 8% (w/v).

DISCUSSION

In the present study, we evaluated the antifungal activity of the extracts of 27 plant species against early blight pathogen (*A. solani*). Leaf extract of *L. nobilis* (4%) was highly effective in reducing the radial growth of *A. Solani*. At some concentrations, extracts from *S. officinalis* (leaf), *H. lupulus* (flower bud), *C. arvense* (leaf) and *S. officinalis* also inhibited the mycelial growth of the fungus over 50%. Similar effect of other various plant extracts effective against *Alternaria* spp. have been reported by several workers (Hassanein et al., 2008; Abd-El-Khair and Haggag, 2007; Muto et al., 2005; Patil et al., 2001; Srivastava et al., 1997). The aqueous neem leaf extracts inhibited the mycelial growth of *A. solani* (Hassanein, et al., 2008). Muto et al. (2005) showed that the extracts derived from potato sprouts and *Solanum nigrum* roots showed complete inhibition of conidial germination of *Alternaria brassicicola* at a concentration of 10% (w/v) from fresh tissues, and 1% (w/v) from dry tissues. Also, the ethanol extracts from dry fruit tissues of *S. nigrum* completely inhibited conidial germination of the fungus. Vijayan (1989) reported that the bulb extract of *Allium sativum*, leaf extract of *Aegle marmelos* and flower extract of *Catharanthus roseus* inhibited the spore germination and mycelial growth of *A. solani*. The bulb extract of *A. sativum* inhibited the mycelial growth of *Alternaria helianthi* (Sivagami, 2003). Similarly, Mishra et al. (2009) reported that the complete inhibition (100%) of spore germination in *A. solani* was observed with chloroform and acetone extract of *Cinnamomum zeylanicum* bark as well as with petroleum ether and ethanol extracts of *C. zeylanicum* leaf at the lowest concentration (50 µg/ml).

Nineteen out of twenty seven plant extracts accounted for less than 50% inhibition of mycelial growth of *A. solani* at a concentration of 4% (w/v). In contrast, extracts from leaves of *D. consolida*, *C. segetum*, and *C. maculatum* were ineffective in inhibiting mycelial growth of *A. solani* isolate AS-5. Evaluation of *L. nobilis*, *S. officinalis*, *H. lupulus* and *C. arvense* extracts further with increasing concentration of the extracts up to 8% (w/v) exhibited increased inhibitory properties of the extracts up to 100%. This is in conformity with the finding of Abd-El-Khair and Haggag (2007) who observed that higher concentration of plant extracts induced maximum inhibition in fungal growth. Similar observations have been reported by Farcasanu and Oprea (2006) who found that doubling the *S. officinalis* leaf extract concentration (100 µl/ml), inhibited cell growth of yeast almost completely. The effective antifungal nature of *L. nobilis*, *S. officinalis*, and

H. lupulus and *C. arvense* extracts is due to the presence of various compounds in these plants. In previous studies, it was reported that antifungal property of *H. lupulus* depended mainly on the concentration of secondary metabolites, phenol substances and flavonoids, especially alpha and beta acids, prenylflavonoids and proanthocyanidins (Stevens et al., 1997; Taylor et al., 2003; Ūrgeova and Polivka, 2009).

In conclusion, the results obtained from this study shows that the methanolic extracts of *L. nobilis*, *S. Officinalis*, *H. lupulus* and *C. arvense* used in this study exhibit antifungal effect on *A. solani*. So these extracts could be useful in the treatment of fungal infections caused by *A. solani*.

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