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Full Length Research Paper

In vitro antifungal activities of 26 plant extracts on mycelial growth of *Phytophthora infestans* (Mont.) de Bary

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Antifungal activities of 26 plant extracts were tested against *Phytophthora infestans* using radial growth technique. While all tested plant extracts produced some antifungal activities *Xanthium strumarium, Lauris nobilis, Salvia officinalis* and *Styrax officinalis* were the most active plants that showed potent antifungal activity. They totally inhibited the mycelial growth of *P. infestans*. The other tested plant extracts exhibited moderate activity and average daily radial growth of fungus varied from 0.8 to 5.0 mm/day which were significantly lower than the control. The lowest antifungal activity was observed on *Cynodon dactylon* extract. The minimum inhibitory concentration (MIC) of the extracts ranged between 2 and 8% (w/v). *X. strumarium* extract produced the lowest MIC value of 2% which was lower than the standard fungicide Ridomil Gold mz 68 WP. 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, *Phytophthora infestans*, antifungal, minimum inhibitory concentration (MIC).

INTRODUCTION

Potato is one of the important crops in whole world due to its high value for human nutrition (Desjardins et al., 1995; FAO, 2010). It is the fourth mosts imported crop and is planted in 18.2 million hectare with a total yield reaching 314.1 million tone (FAO, 2010). Many insect and diseases attack foliage and tubers of potato during growing season and after harvesting in storage. Late blight is a devastating disease of potato, reducing crop quantity and quality (Fry et al., 1993). Many varieties in use today are moderately or extremely susceptible to blight such that fungicide application is a widely implemented strategy to control the disease. However, the chemical control of the disease has several drawbacks. During the late 1980s and 1990s, introduction of new clonal lineages of *Phytophthora infestans* to potato growing areas of the world led to severe late blight outbreaks (Fry and Goodwin, 1997a,b; Inglis et al., 1996). These new clonal lineages caused a new disease management challenge because many were resistant to the fungicide metalaxyl, which had become an integral tool for foliar late blight suppression (Bashan et al., 1989; Daayf and Platt, 1999, 2000; Daayf et al., 2000), and increased the costs of crop production. Moreover, the public has expressed concerns about the heavy reliance on chemicals in plant protection strategies. Therefore, developing a new control strategy to prevent development of new clonal lineages and to meet public demand in reduction of pesticide use is needed.

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Abbreviations: PDA, Potatoe dexyrose agar; **MIC,** minimum inhibitory concentration.

Extracts of many higher plants are reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory and field tests. 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). Controlling of microorganism originated plant disease with plant extracts as components in integrated pest management strategy has been tested by many scientists since 1990s. Blaeser and Steiner (1999) reported that *Potentilla eracta* and *Salvia officinalis* extracts exhibited high antifungal activities against *P. infestans* on tomato plants under greenhouse conditions. Late blight disease severity was significantly lowered by *Malvae folium, Salviae folium* (from *S. officinalis*) and *Bardanae radix* (from *Arctium lappa)* (Krebs and Forrer, 2001). Muto et al. (2005) tested the extracts derived from fresh and dry tissues of 14 plant species against *P. infestans* and *Alternaria solani*. Suspensions and extracts of medicinal plants reduced foliar blight of potatoes (*P. infestans)* significantly in wet room experiments (Krebs et al., 2006).

In this study, 26 plant extracts with antifungal properties against *P. infestant* were tested under laboratory conditions to determine the effect of these extracts on mycealial growth of the fungus and determine minimum inhibitory concentration.

MATERIALS AND METHODS

Source of pathogen

The pathogenic isolates of *P. infestans* (TPI-2), cultured from diseased potato leaf was used as inoculum. The pathogen isolate was maintained in V8 juise agar medium throughout the study at 25 ± 2°C in an incubator for 7 day.

Plant materials and extract preparation

The extracts of twenty six naturaly growing plant species (Table 1) were used in the present study. The plants were collected during spring and summer of 2002 to 2003 from different localities of Taşliçiftlik, 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* collected from Mersin. The plant parts (leaves and fruits) were air dried at room temperature for three weeks in the dark conditions. The dried plants 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 of the powdered, dried 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 flaks were closed with a cotton balls and covered with aluminum foil and then placed on a horizontal shaker (HS 260 Basic, IKA Group) and shaked 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 methanol were evaporated 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. These stock suspensions were stored at 4°C and used within four days.

Determination of antifungal properties of the extracts

The antifungal properties of the extracts were tested using the radial growth method as described in Banso et al. (1999). Potatoe dextrose agar (PDA) medium was prepared by autoclaving at 121°C and cooled to 45°C. Afterwards, appropriate quantities of stock solution of each extract and distilled water were added to PDA medium to get 4% (w/v) concentrations of the extracts in the medium. In the control, 10% acetone (v/v) water mixture was added to PDA to get 4% (w/v) concentration in the medium. The plant extracts were thoroughly mixed with the medium. Twenty milliter of each medium was poured in each 90 mm diameter sterilized Petri plates and left to solidified over night. Mycelial discs of 5 mm diameter were taken from 7 days old *P. infestans* 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 use as references for recording growth. Plates were incubated at 25 ± 2°C for 7 days. Radial growth along each line was recorded at exactly 24 h intervals using callipers (Mitutoyo). Each treatment was replicated four times. The whole experiment was repeated three times.

Determination of minimum inhibitory concentration (MIC)

Based on effects of plant extracts on radial growth experiment, *Glycyrrhiza glabra, Lauris nobilis, S. officinalis, Solanum nigrum, Sytrax officinalis* and *Xanthium strumarium* extracts were used for further determination of minimum inhibitory concentration. 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 solition of each extract and distilled water to PDA medium and thoroughly mixed with the medium. Twenty milliliter of each medium was poured in each 90 mm diameter sterilized Petri plates. Plates were inoculated, incubated and evaluated as described earlier. Each treatment was replicated four times. The whole experiment was repeated twice. Ridomil Gold mz 68 WP (Metalaxyl 4% + Mancuzeb 64%) was used as a standard, synthetic fungicide for comparison of results under identical conditions. Minumum inhibitory concentration was used to determine the concentration at which no visible mycelial growth was observed after incubation period.

Statistical analysis

Radial growth data were subjected to variance analysis using MINITAB software programe Release 14 (McKenzie and Goldman 2005). Analysis of variance ($\propto$ = 0.05) was carried out on growth rates (mm/day) and it was followed by comparison of means of growth rates using the Duncan's multiple range test ($\propto$ = 0.05).

RESULTS

Determination of antifungal properties of the extracts

The results of *in vitro* antifungal activity of 26 plant extracts are summarized in Table 1. The extracts produced different levels of antifungal activity against *P. infestans*. Results indicated that all the extracts significantly reduced the radial growth of *P. infestans*, in comparison

Table 1. Antifungal effect of 26 plant extracts on radial mycelial growth of *P. infestans* (average radial growth/day) at 4% (w/v) extract concentration.

Family	Plant species	Plant part	ARG ± SD (mm/day)*
Asteraceae	*Xanthium strumarium* L.	Fruit	0.0±0.0[a]
Lauraceae	*Laurus nobilis* L.	Leaves	0.0±0.0[a]
Lamiaceae	*Salvia officinalis* L.	Leaves	0.0±0.0[a]
Styracaceae	*Styrax officinalis* L.	Fruit	0.0±0.0[a]
Fabaceae	*Glycrrhiza glabra* L.	Fruit	0.8±0.9[b]
Solanaceae	*Solanum nigrum* L.	Fruit	1.0±0.0[bc]
Caprifoliaceae	*Sambacus nigra* L.	Fruit	1.1±0.1[bc]
Cannabinaceae	*Humulus lupulus* L.	Flower bud	1.3±0.1[c]
Asteraceae	*Chrysanthemum segetum* L.	Leaves	1.8±0.9[d]
Asteraceae	*Circium arvense* (L.) Scop.	Leaves	1.9±0.4[d]
Apocynaceae	*Nerium oleander* L.	Leaves	2.2±0.2[de]
Asteraceae	*Artemisia vulgaris* L.	Leaves	2.4±0.1[ef]
Araliaceae	*Hedera helix* L.	Leaves	2.5±0.3[ef]
Rubiaceae	*Rubia tinctoria* L.	Leaves	2.6±0.2[ef]
Ranunculaceae	*Delphinium consolida* L.	Leaves	2.7±0.3[fg]
Solanaceae	*Datura stramonium* L.	Fruit	2.7±0.1[fg]
Cucurbitaceae	*Ecballium elaterium* (L.) A. Rich.	Fruit	2.8±0.3[fg]
Poaceae	*Lolium temulentum* L.	Leaves	3.1±0.1[gh]
Chenopodiacea	*Chenopodium album* L.	Leaves	3.4±0.1[h]
Poaceae	*Sorghum halepense* (L.)	Fruit	4.1±0.1[ı]
Asteraceae	*Arctium lapa* L.	Leaves	4.3±0.2[ı]
Scrophulariaceae	*Verbascum songaricum* L.	Leaves	4.3±0.1[ı]
Guttiferae	*Hypericum perforatum* L.	Flowers	4.8±0.1[j]
Apiaceae	*Conium maculatum* L.	Leaves	4.8±0.4[j]
Rubiaceae	*Galium aperina*	Leaves	5.0±0.4[j]
Poaceae	*Cynodon dactylon* L.	Leaves	6.0±0.4[k]
Control (PDA with 10% acetone)			6.4±0.1[k]

*Means followed by standard error with same letter are not significantly different (P < 0.05). ARG, Average radial growth, SD, standart deviation.

with the control, except, *Cynodon dactylon* extract at 4% concentration. The highest inhibition of mycelial growth of *P. infestans* was observed with *X. strumarium, L. nobilis, S. officinalis,* and *Sy. officinalis* extracts. These plants extract totally inhibited the mycelial growth of fungus during 7 days of test period. In terms of antifungal activities, they were followed by *G. glabra, S. nigrum, Sambacus nigra* and *Humulus lupulus* extracts, daily average mycelial growth ranged from 0.8 to 1.3 mm/day, respectively for these extracts. The remaining 17 plant extracts produced moderate antifungal activities and daily radial growth of fungus grown on PDA contaning these extracts varied between 1.8 and 5.0 mm (Table 1).

Determination of MIC

The MIC of five plants extracts is presented in Table 2. The antifungal activity of the extracts was enhanced by increase in the concentration of the extracts. Among the tested extracts, *L. nobilis* and *S. officinalis* extracts exhibited the complete mycelial growth inhibition at 4% extract concentration as it was obseved in radial growth test. Extract of *X. strumarium* showed the lowest MIC against *P. infestans* at 2% extract consentration. The extracts of *G. glabra, S. nigrum* and *Sy. officinalis* showed highest MIC against *P. infestans* with a concentration of 8% (w/v) after 7 day of incubation. MIC of *X. strumarium* is lower than the recommended concetration of synthetic fungiside Ridomil Gold mz 68 WP (Metalaxyl 4% + Mancozeb 64%).

DISCUSSION

Potato late blight (*P. infestans*) is the major disease affec-ting potato production (Kapsa and Koodziejczyk, 2005). The development of disease resistance to conventional fungicide and environmental contamination problems creates pressure on growers to adopt new control

Table 2. Minimum inhibitory concentration of six plant extracts against *P. infestans.*

Plant species	Minimum inhibitory concentration (% w/v)
Glycyrrhiza glabra	8.0
Lauris nobilis	4.0
Salvia officinalis	4.0
Solanum nigrum	8.0
Sytrax officinalis	8.0
Xanthium strumarium	2.0
Metalaxyl 4% + Mancuzeb 64%	2.5

strategy in potato production (Gamliel and Yarden, 1998). Additionally, public demand to minimize the pesticide residues in the marketable products of potatoes forces growers and chemical companies to develop safer chemical compounds than today's marketed agents. Therefore, biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies. Exploitation of naturally available chemicals from plants, which retards the reproduction and growth of plant pathogenic fungi, would be a more realistic and ecologically sound method for integrated plant disease management and will have a prominent role in the development of future commercial pesticides for crop protection strategies, with special reference to the management of plant diseases (Varma and Dubey, 1999; Gottlieb et al., 2002). Several reports mentioned that the plant extracts play an important role in controlling the late blight pathogens *in vitro* and *in vivo* (Krebs et al., 2006; Stephan and Koch, 2002; Stephan et al., 2005; Blaeser and Steiner, 1999; Ashrafuzzaman et al., 1990; Gamliel and Yarden, 1998). Considering these as a first step in the present investigation, 26 plants were screened *in vitro* for antifungal activities against *P. infestans*. These plants were selected based on literatures and random choosing from the local flora. The screening revealed that *X. strumarium, L. nobilis, S. officinalis* and *Sy. officinalis* (leaf or fruit) extracts completely inhibited mycelial growth of *P. infestans* at 4% concentration. These results are in agreement with previous studies showing the antifungal activitiy of leaf or fruit extracts of these plant species to *Phytophthora* species (Dorn et al., 2007; Al Azeez and Nezam, 2009; Kim et al., 2002; Blaeser et al., 2002). In a similar study, Kim et al. (2002) reported that extracts of cocklebur (*X. strumarium*) effectively inhibited the mycelial growth and germination of zoospores of *Phytophthora drechsleri*. The remaining plant extracts have differrent degrees of inhibitory effects on mycelial growth of the pathogen fungus. In previous studies, efficacy of plant extracts against *P. infestans* has been demonstrated by several workers (Latten, 1994; Meinck, 1999; Neuhoff et al., 2002; Rohner et al., 2004). Blaeser et al. (2002) reported that the extracts of *S. officinalis* reduced foliar blight and increased the yield of potato up to 12 to 17%. According to Blaeser et al. (2002), extracts of *S. officinalis* inhibited the germination and motility of zoospores. In another study, Khair and Haggag (2007) evaluated the antifungal efficacy of several plant extracts against *P. infestans* and they reported that lemon grass (*Cymbopogon citratus*) leaves extract gave the best result to inhibit the spore germination and reduce the mycelial growth. Lowest MIC value of 2% was obtained with *X. strumarium* fruit extract and proved to be as effective as the recommended Ridomil Gold concentration, which had been included in the experiments for comparison. On the other hand, *G. glabra*, *S. nigrum* and *S. officinalis* extracts exhibited the highest MIC values (8%). The minimum inhibitory concentration values of the plant extracts against *P. infestans* showed that fungi vary widely in the degree of their susceptibility to antifungal agents.

In particular, *X. strumarium* extract appear to be very active against the fungal disease and could be used as potent biocide to treat late blight disease in plants caused by *P. infestans* as it showed maximum activity even at lower concentration which is lower than the standard antifungal agent, Ridomil Gold mz 68 WP (Metalaxyl 4% + Mancozeb 64%). The finding of the present investigation is an important step towards isolation and characterization of the antifungal agent and its further evaluation for crop protection strategies.

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