IN VITRO AND *IN VIVO* ACTIVITY OF SELECTED PLANT CRUDE EXTRACTS AND FRACTIONS AGAINST *PENICILLIUM ITALICUM*

Ghassan Jadou Mousa Kanan, Rasha Abdel-Wahab Khaleel Al-Najar*

Department of Biological Sciences, Mu'tah University, Karak – Jordan, P. O. Box (7)

Received: February 4, 2009
Accepted: September 10, 2009

Abstract: The objective of this study is to evaluate (*in vitro* and *in vivo*) seven plant extracts and their liquid fractions against four *Penicillium italicum* isolates. The *in vitro* study revealed that a concentration 520 μg/ml of crude extract of sticky fleabane leaves or cinnamon bark generated maximum percentage inhibition of 54% and 43%, respectively, against tested fungal isolates. A concentration of 130 μg /ml from each extract (except harmal and garlic where, 390 μg/ml were required) caused complete inhibition of fungal growth of isolates Pi.1 and Pi.3 infecting orange fruit. A concentration of 130 μg/ml of nightshade fruit, fenugreek or sticky fleabane extract inhibited completely the growth of isolates Pi.3 and Pi.5 infecting lemon fruits, whereas a concentration of 390 μg/ml was required to inhibit the growth of isolate Pi.1. Methanolic fractions of cinnamon, garlic or sticky fleabane completely inhibited the growth of fungal isolates. The IC_{50} values for these fractions were found to be in the range of: 11.2–24; 30.25–31.50; 25.0–36.0 μg/ml, respectively. A concentration 20 μg/ml of cinnamon hexane fraction inhibited the growth of the fungal isolates, with IC_{50} values of 13, 13.75, 14 and 13 μg/ml, respectively, obtained against isolates Pi.1, Pi.3, Pi.5 and Pi.6. The nightshade hexane fraction completely inhibited the growth of isolates Pi.1 and Pi.3 with IC_{50} values of 80 and 37.5 μg/ml, respectively. Cinnamon aqueous fraction completely inhibited the growth of isolates Pi.1 and Pi.5 (IC_{50} were 61.25 and 58.5 μg/ml, respectively).

Key words: blue mould, citrus fruit, plant extracts

INTRODUCTION

The filamentous fungus *Penicillium italicum* Wehmer (blue mould) causes a universal post-harvest disease of almost all kinds of citrus fruit (Prusky *et al.* 2004). Economic losses caused by post-harvest pathogens are greater than is often realized and the avoidable losses between the farm gate and the consumer are of considerable concern (Soylu *et al.* 2005). However, consumers' demand for pesticide-free food and the development of pathogen strains resistant to currently used fungicides, in addition to the ineffectiveness of such pesticides, necessitate the development of environmentally safer control options for post-harvest diseases (Hammer *et al.* 1999). Plant extracts and their essential oils are one of several non- synthetic chemical control options that have recently received attention for controlling plant diseases (Soylu *et al.* 2005; Abad *et al.* 2007). The methanolic extract of fenugreek was potent in inhibiting dermatophytes and *Candida albicans* (Shtayeh and Abu Ghdeib 1999; Olli and Kirti 2006). Extracts of harmal seeds and roots were found to contain a mixture of active alkaloids, and among these harmaline was the most active antifungal agent (Telezhenetskaya and Dyakonov 2004). The chemical constituents of garlic bulb had shown inhibitory activity against several microbes including bacteria, fungi and viruses (Yoshida *et al.* 1987; Elsom 2000). The antifungal activity of garlic is attributed to the main biologically active component of garlic extract termed allicin (Marino *et al.* 2001). Similarly, an allicin derivative, the compound ajoene, has shown antifungal activity against *Aspergillus niger*, *C. albicans* and *Paracoccidioides brasiliensis* (Gurib-Fakim *et al.* 2005). Concerning the activity of cinnamon bark constituents, eugenol as well as cinnamaldehyde have consistently been reported to have antifungal activity (Delaquis *et al.* 2002). Extensive studies were conducted to elucidate the nature and the biological activity of sticky fleabane extracts (Wang *et al.* 2004). All types of sticky fleabane extracts proved to have significant antifungal activity where the oily leaf paste made with organic solvents exhibited the greatest *in vitro* antifungal efficacy against dermatophytes, *Candida* spp and downy mildew (Cafarchia *et al.* 2002; Cohen *et al.* 2006). Furthermore, a sesquiterpene lactone isolated from *I. viscosa* flowers possessed *in vitro* efficacy against *Microsporum canis*, *M. gypseum* and *Trichophyton mentagrophytes* (Abu Zarga *et al.* 1998; Cafarchia *et al.* 2002). The herbaceous annual plant nightshade, due to its high content of steroidal alkaloids, showed antifungal activity against 11 agronomically important fungi including: *Aspergillus* spp, *Rhizopus* spp, *Fusarium* spp, *Mucor mucedo, Bipolaris oryzae* and *Rhizoctonia solani* (Muto *et al.* 2006; Zhou *et al.* 2006; AL-Fatimi *et al.* 2007). The current investigation aimed at *in vitro* and *in vivo* evaluation of seven plant extracts and their fractions for the control of *P. italicum*. Plant extracts were obtained from

*Corresponding address:
gkanan@mutah.edu.jo

fenugreek seeds (*Trigonella foenum-graecum* L.), harmal seeds (*Peganum harmala* L.), garlic cloves (*Allium sativum* L.), cinnamon bark (*Cinnamomum cassia* L.) Presl, sticky fleabane leaves (*Inula viscose* L.) Aiton, nightshade leaves and fruit (*Solanum nigrum* L.).

MATERIALS AND METHODS

***Penicillium italicum* isolates tested**

Conidiospores of four *P. italicum* isolates (Pi.1, Pi.3, Pi.5, and Pi.6) were obtained from decayed orange (*Citrus sinensis* L.), lemon (*Citrus limon* L.) and calamondin (*Citrus mitis* L.) fruit.

Citrus fruit types used

Two citrus fruit types were used: orange (*C. sinensis* L.) and lemons (*C. limon* L.).

Plant materials analysed

Crude extracts of only six out of seventy plant species tested have shown antifungal activity against isolates of *P. italicum*. These are: fenugreek seeds (*Trigonella foenum-graecum* L.), harmal seeds (*Peganum harmala* L.), garlic cloves (*Allium sativum* L.), cinnamon bark (*Cinnamomum cassia* L.), sticky fleabane leaves (*Inula viscosa* L.) and nightshade leaves and fruit (*Solanum nigrum* L.). The former four plant materials were brought from traditional medicine shops in Irbid city, whereas, the latter two were collected from wild-type populations occupying orchard fields and roadsides of Mu'tah and Al-Iraq towns within the Al-Karak area in Jordan.

Media

Aspergillus nidulans complete medium (CM) described by Cove (1966) was used [it gave maximum zone of growth as compared to potato dextrose agar (PDA) media] with slight modification (i.e. pH 6, supplemented with 10 mM proline) in order to achieve optimal growth conditions for various isolates of *P. italicum*.

Purification of fungal isolates

Conidiospore suspensions in a 5 ml physiological saline/Tween 80 (0.05%) solution were harvested from each isolate to give a concentration of approximately 1×10^8 spores per milliliter. An aliquot of 100 µl from a dilution of 10^{-6} or 10^{-7} was plated again on complete media to confirm purity and identity of the culture (Zhang *et al.* 2004).

Optimal growth conditions of fungal isolates

Nine replicates (for each condition tested) of conidiospore suspensions (20 µl) from each tested isolate were inoculated into complete media, having different pH regimes (i.e. 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 9.0) for optimal pH testing. At pH 6.0 (optimal pH), L-proline at a concentration of 10 mM was the best nitrogen source used among the following compounds: NH_4^+, NO_3^-, Urea; L-proline, L-lysine, L-arginine, L-adenine, L-glutamine, and L-histidine. In addition, inoculated plates of complete media adjusted to optimal pH 6.0, and supplemented with 10 mM proline were incubated at five temperature regimes (i.e. 10°C, 20°C, 25°C, 30°C, 37°C) in order to determine the optimal temperature of growth. Also, various carbon sources (glucose, sucrose, sorbitol, fructose, and maltose) were tested at a final concentration of 10 g/l to determine the best carbon source (glucose). Each group of nine replicates were incubated for 5 days, at 25°C (optimal temperature) or at the tested temperature, and the radius of each growing colony measured in two directions at right angles to each other.

Preparation of extracts

Plant material was dried in the shade, ground to a fine powder in liquid nitrogen and extracted (48 h) with absolute ethanol in a soxhlet apparatus (Ndukwe *et al.* 2006). The solvent was removed using rotary evaporator (Heidolph, VV2000) under reduced pressure at temperatures below 50°C. The resulting crude extracts were stored at –20°C until assayed. Stock solutions and serial dilutions of extracts and fractions were prepared in dimethylsulphoxide (DMSO) (Ambrozin *et al.* 2004). Control experiments were performed using DMSO at the same concentration as used to test the extracts. Extracts were dissolved in DMSO and evaluated for their ability to inhibit the growth of *P. italicum* isolates.

Fractionation of crude plant extracts

Each crude extract sample was fractionated with a 1:1 ratio of water /dichloromethane (v/v). The resultant aqueous fraction was further extracted with dichloromethane, concentrated to dryness using rotary evaporation and stored in sterile containers at 4°C until used. The dichloromethane fraction was concentrated to dryness using rotary evaporation, and diluted with n-hexane/90% methanol (1:1). The hexane and methanol fractions were concentrated to dryness using rotary evaporation and kept in sterile containers at 4°C until used. Each fraction was dissolved in (v/v) dimethylsulphoxide (Souza-Fagundes *et al.* 2002).

Surface sterilization of citrus fruit

After preliminary washing under running tap water for 5 minutes, fruit was washed with 6% sodium hypochlorite solution by shaking and rubbing with a paint brush. This was followed by immersion in sterile distilled water for two minutes, surface sterilization in 70% ethanol for another two minutes and finally placing inside a laminar flow cabinet.

Antifungal activity assay by the agar well diffusion method

An aliquot of 100 µl spore suspension ($1x10^8$ spores/ml) of each isolate was streaked in radial patterns on the surface of complete media plates. Wells of 6 mm diameter were made in the medium and each was filled with a certain concentration (0.65, 1.3, 13, 32, 65, and 97 µg) of crude extract. DMSO was used as a control for the ethanolic extracts. The cultured plates were incubated for 3–5 days at 25°C. The zone of inhibition was measured in two directions at right angles to each other. Experiments were carried out with three replicates per treatment and each treatment was repeated at least twice (Ndukwe *et al.* 2006).

Antifungal assay of crude extracts and their fractions by amended agar method

Each crude extract was fractioned to provide three fractions, aqueous, hexane and methanolic. Also the emulsion was tested that may sometimes form between layers. Stock solutions of each fraction were filter sterilized through a 4 μm Millipore filter (Soylu *et al.* 2005). Each concentration (130, 260, 390 and 520 μg/ml) of the crude extracts or their fractions (5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μg/ml) from plant species were amended on the surface of solidified complete media. 20 μl of conidiospore suspension ($1x10^8$) from each of the isolates were pipetted and left as drops on the surface of the amended media and incubated at room temperature for at least one hour until the liquid became completely absorbed. Three inocula per isolate/plate and three replicate Petri plates were used per treatment and each treatment was repeated at least twice. Along with each treatment, 20 μl dimethylsulfoxide (DMSO) were replicated as above and used as controls for the ethanolic extracts. The inoculated Petri dishes were incubated for 3–5 days at optimal temperature (25°C). Colony diameter was determined by measuring the average radial growth of each tested isolate. The radius of the growing colonies was measured in two directions at right angles to each other. The Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration of the extract inhibiting the visible growth of each isolate (Obagwu and Korsten 2003).

Determination of sensitivity of fungal strains to plant crude extracts or fractions

The percentage of mycelial growth inhibition by each extract or fraction concentration was calculated from the mean colony diameter (mm) on medium without plant fraction (control) and from the mean colony diameter (mm) on each fraction amended plate (zone of growth). A linear regression of percent inhibition versus plant fraction concentration estimated to produce 50% growth inhibition (IC_{50}) was determined from the regression equation or by interpolation from the regression line. Percentage inhibition of mycelial growth was determined using the following formula (Nwachukwu and Umechuruba 2001).

$$\% \text{ MGI} = \frac{\overline{x} - xi}{\overline{x}} \times 100\%$$

where:
% MGI denotes: % of mycelial growth inhibition
$\overline{x}$: mean diameter (mm) of control colony on non-amended medium
xi: mean diameter (mm) of tested colony replicates on a single crude extract or fraction amended plate (zone of growth).

Fruit inoculation with conidiospores and plant extracts

Fruit was wounded (2-wounds per fruit) at the equatorial side with a sterile stainless steel scalpel where each wound was about 4 mm long and 2 mm deep (Zhu *et al.* 2006). 15 μl of conidiospore suspension from each isolate were inoculated under aseptic conditions into each wound using a micropipette. Two hours later, each wound was inoculated with a pre-determined concentration from each plant extract. Control fruit was subjected to the same treatments except that sterile distilled water was used instead of plant extract. At least two replicates were used for each treatment and each test was repeated twice. The treated fruits were labeled, sealed in moistened sterile transparent nylon bags and incubated at 22°C to 25°C for two weeks before assessing decay or fungal growth symptoms.

Statistical analysis

The concentration of plant crude extract or fraction producing 50% growth inhibition (IC_{50}) was calculated by regression analysis to determine the relationship between the size of inhibition zone (mm) and the concentration (μg) of crude extract or fraction (log value) using Microsoft Excel 2003 and SPSS program version 10.

RESULTS

Sensitivity of *P. italicum* isolates to plant crude extracts as determined by the agar well diffusion method

Results of regression analysis for the relationship between the size of inhibition zone (mm) and plant extract concentration (log value) indicated that there was a significant correlation between the concentrations of cinnamon extract used and the average inhibition zones for the four isolates (Pi.1, Pi.3, Pi.5 and Pi.6) of *P. italicum*. The correlation coefficient values observed for these fungal isolates were: $r = 0.878^*$, $p = 0.022$; $r = 0.877^*$, $p = 0.022$; $r = 0.842^*$, $p = 0.036$, and $r = 0.879^*$, $p = 0.021$, respectively. A concentration of 97 μg/ml cinnamon extract resulted in mean inhibition zones of 24.83, 23, 20.17, and 18.83 mm for the four fungal isolates, respectively. However, the results indicated that the range of concentrations from 0.65 μg/ml to 97 μg/ml nightshade fruit or leaves, fenugreek seeds or sticky fleabane leaf extracts showed no inhibitory effect on the growth of four *P. italicum* isolates (i.e. zero zone of inhibition).

Sensitivity of *P. italicum* isolates to plant crude extracts as determined by amended agar method

The data presented in table 1 show that the concentrations of sticky fleabane extract tested were significantly correlated [at the 0.01 and 0.05 levels of significance] with the percentages of inhibition zones in isolates Pi.1, Pi.3, Pi.5 and Pi.6. Sticky fleabane leaves followed by cinnamon bark extract were observed to be the most effective against *P. italicum* isolates. Whereas 520 μg/ml sticky fleabane extract resulted in hyphal growth inhibition values of 54.09%, 53.73%, 52.85% and 46.32% for isolates Pi.3, Pi.5, Pi.6 and Pi.1, respectively, the the same concentration (i.e. 520 μg/ml) of cinnamon bark extract gave percentage inhibition values of 24.59, 35.64, 43.53 and 41.59. Regarding the effect of the remaining plant materials (Table 1), fungal isolates Pi.6 and Pi.5 were observed to be more susceptible (i.e. showed the highest % of fungal growth inhibition) to extract activity than isolates Pi.1 and Pi.3 with 520 μg/ml of extract type. An exception to this pattern was the nightshade leaf extract.

Table 1. Sensitivity of four *P. italicum* isolates to different concentrations of various plant crude extracts tested by amended agar method (*in vitro*)

Source of plant crude extracts[a]	Conc. range [μg][b]	Mean size of growth zone [mm]±SD (range)[c]	Inhibition (range)[d] [%]	Corr Value (r)[e]	Sig[f] value	Regression equation[g]	Fungal isolate
Nightshade fruits	130–520	30.67±0.82–29.50±9.90	3.17–6.85	0.947	0.153	y = 0.01x+2.91	Pi1
	130–520	29.67±2.16–26.00±0.52	2.73–14.75	0.887	0.113	y = 0.029x+1.39	Pi3
	130–520	25.5±0.55–24.5±1.3	18.62–21.81	0.976*	0.024	y = 0.008x+17.82	Pi5
	130–520	29.5±2.7–22.17±2.14	8.30–31.10	0.958*	0.042	y = 0.054x+3.38	Pi6
Nightshade leaves	130–520	24.5±0.55–22.33±0.82	22.64–29.48	0.994*	0.006	y = 0.018x+20.54	Pi1
	130–520	24.83±0.41–20.67±0.82	18.58–32.24	0.894	0.106	y = 0.032x+16.94	Pi3
	130–520	23.5±1.38–19.17±1.47	24.99–38.83	0.923	0.077	y = 0.035x+22.61	Pi5
	130–520	20.67±1.03–19.5±1.65	35.76–39.39	0.799	0.201	y = 0.010x+33.17	Pi6
Cinnamon bark	130–520	25.67±0.52–18.5±0.84	18.96–41.59	0.996*	0.004	y = 0.056x+11.85	Pi1
	130–520	27.33±0.82–23±0.55	10.39–21.59	0.923	0.077	y = 0.034x+8.197	Pi3
	130–520	23.5±1.98–20.17±2.32	25.00–35.64	0.983*	0.017	y = 0.027x+21.01	Pi5
	130–520	23.17±0.98–18.17±1.84	27.99–43.59	0.809	0.191	y = 0.036x+27.47	Pi6
Garlic cloves	130–520	25.17±0.41–25.17±0.75	18.90–20.53	0.913	0.087	y = 0.004x+18.69	Pi1
	130–520	22.5±0.84–21±0.63	26.23–31.15	0.563	0.437	y = 0.011x+23.22	Pi3
	130–520	22.67±0.82–20.5±1.23	27.66–34.57	0.766	0.234	y = 0.015x+27.66	Pi5
	130–520	22.5±1.64–20.67±0.82	30.06–35.76	0.938	0.062	y = 0.015x+27.21	Pi6
Sticky fleabane leaves	130–520	21.67±0.52–17±0.0	31.59–46.32	0.993**	0.007	y = 0.036x+26.85	Pi1
	130–520	20.67±0.52–14±0.63	32.24–54.09	0.983*	0.017	y = 0.053x+25.41	Pi3
	130–520	21±1.79–14.5±2.17	32.98–53.73	0.986*	0.014	y = 0.052x+27.66	Pi5
	130–520	18.33±1.63–15.17±0.41	41.97–52.85	0.972*	0.028	y = 0.028x+37.57	Pi6
Fenugreek seeds	130–520	25±0.0–23.5±1.76	21.06–25.79	0.947	0.053	y = 0.013x+18.69	Pi1
	130–520	25.5±1.05–22.5±1.05	16.39–26.23	0.947	0.053	y = 0.028x+13.11	Pi3
	130–520	23.67±1.03–21.5±1.05	24.47–31.38	0.920	0.080	y = 0.019x+20.74	Pi5
	130–520	25.83±0.98–23.33±1.21	19.69–27.47	0.954*	0.046	y = 0.020x+17.88	Pi6
Harmal seeds	130–520	25±0.0–23.5±1.76	21.06–25.79	0.933	0.067	y = 0.011x+20.27	Pi1
	130–520	25.67±1.21–24±1.27	15.85–21.31	0.966*	0.034	y = 0.014x+14.48	Pi3
	130–520	22.17±0.75–22±0.89	29.25–29.79	0.359	0.641	y = 0.003x+27.93	Pi5
	130–520	23±2.09–22.33±1.03	28.51–30.58	0.939	0.061	y = 0.006x+27.73	Pi6

[a] six plant crude extracts were tested against four fungal isolates
[b] a range of concentrations (130, 260, 390, 520 μg/ml) of crude extracts was used from each plant type against tested isolates of *P. italicum*
[c] mean diameter (mm) of control colony (zone of growth) on non-amended medium for isolate Pi1 = 31.67±0.82; Pi3 = 30.5±0.84; Pi5 = 31.33±1.86; Pi6 = 32.17±1.94
[d] % of mycelial growth inhibition was determined from the mean diameter (mm) of control colony on non-amended medium and that tested on amended plate (zone of growth)
[e] correlation coefficient values
[f] level of significance at the 0.05 and 0.01 levels (2-tailed)
[g] regression analysis for the relationship between the size of inhibition zone (mm) and the concentration [μg] of crude extract or fraction (Log-value) . Values are means of six replicates and each experiment was repeated at least twice
* correlation is significant at the 0.05 level (2-tailed)
** correlation is significant at the 0.01 level (2-tailed)

Sensitivity of *P. italicum* isolates to plant crude extract fractions

The results of regression analysis are presented in table 2. These data indicate that the concentrations of cinnamon and garlic methanolic fractions tested showed significant correlation (at either the 0.01 or the 0.05 level of significance) with the percentages of hyphal growth inhibition for *P. italicum* isolates Pi.1, Pi.3, Pi.5 and Pi.6. 20 μg/ml cinnamon methanolic fraction completely inhibited the growth of isolate Pi.1 whereas similar levels of inhibition were obtained at a concentration of 30 μg/ml for isolates Pi.3, Pi.5 and Pi.6**.** In addition, the IC_{50} values obtained for isolates Pi.1, Pi.3, Pi.5 and Pi.6, were observed to be 11.2, 23.4, 24 and 24 μg/ml, respectively. However, the garlic methanolic fraction completely inhibited the growth of the four isolates at a concentration of 40 μg/ml, with IC_{50} values obtained against isolates Pi.1, Pi.3, Pi.5 and Pi.6 of 31.5, 31.25, 30.5 and 30.25 μg/ml, respectively. The methanolic fraction of sticky fleabane leaves was highly effective against the growth of *P. italicum* isolates**,** where complete inhibition of isolates Pi.1 and Pi.3 growth was achieved at all concentrations tested (Table 2). In contrast, such inhibition was obtained at a concentration of 60 and 80 μg/ml against isolates Pi.5 and Pi.6, and this is reflected by IC_{50} values of 25, 25, 34.5 and 36 μg/ml, respectively. However, none of the concentrations of fenugreek, harmal, nightshade leaves or nightshade fruit methanolic fractions resulted in complete inhibition of fungal growth. In comparison, a concentration of 20 μg/ml cinnamon hexane fraction completely inhibited the growth of the four *P. italicum* isolates and the IC_{50} values obtained for isolates Pi.1, Pi.3, Pi.5 and Pi.6 reached 13, 13.75, 14, and 13 μg/ml, respectively. The hexane fraction of nightshade leaves extract completely inhibited hy-

Table 2. Sensitivity of four *P. italicum* isolates to various fractions of different plant extracts (*in vitro*)

Plant extract fraction	Fungal isolate	Conc [μg]	% of inhibition[a]	IC_{50}[b]	Regression Equation[c]	Corr Value (r)[d]	Sig[e] value
1	2	3	4	5	6	7	8
Cinnamon /Methanol	Pi1	5–10 20–50	37.93–43.10 100	11.2	y = 13.744x+32.1	0.836*	0.038
	Pi3	5–20 30–50	5.56–24.07 100	23.4	y = 23.60x–26.42	0.911*	0.011
	Pi5	5–20 30–50	10.71–16.07 100	24	y = 22.35x–21.07	0.890	0.018
	Pi6	5–20 30– 50	29.31–48.28 100	24	y = 17.49x+6.9	0.920**	0.009
Hexane	Pi1	5–10 20–50	13.79–29.31 100	13	y = 18.37x+9.54	0.842*	0.035
	Pi3	5–10 20–50	12.96–20.37 100	13.75	y = 19.26x+4.81	0.836*	0.038
	Pi5	5–10 20–40	16.07–16.07 100	14	y = 19.18x+4.90	0.828*	0.042
	Pi6	5–10 20–40	17.24–29.31 100	13	y = 17.9x+11.84	0.840*	0.036
Aqueous	Pi1	50–60 70–80	34.48–43.10 100	61.25	y = 25.35x+6.03	0.921	0.079
	Pi3	50–80	46.30–59.26		y = 3.9x+43.52	0.944	0.056
	Pi5	50–60 70–0	39.29–51.79 100	58.5	y = 23.04x+15.18	0.934	0.066
	Pi6	50–80	30.36–48.28		y = 5.89x+25.19	0.995**	0.005
Between fractions	Pi1	50–80	43.11–53.54		y = 3.28x+42.24	0.854	0.146
	Pi3	50–80	27.78–53.71		y = 7.96x+20.37	0.968*	0.032
	Pi5	50–80	41.07–67.89		y = 8.39x+30.36	0.933	0.067
	Pi6	50–80	44.83–58.62		y = 4.48x+38.79	0.943	0.057
Fenugreek/ Methanol	Pi1	50–80	4.444–20.0		y = 5.11x–3.33	0.899	0.101
	Pi3	50–80	0.0–30.44		y = 9.783x–14.13	0.908	0.092
	Pi5	50–80	0.0–11.11		y = 3.33x–3.33	0.939	0.061
	Pi6	50–80	0.0–17.78		y =6x–6.67	0.996**	0.004
Hexane	Pi1	50–80	13.333–26.67		y = 0.422x–9.11	0.933	0.067
	Pi3	50–80	23.91–36.96		y = 0.48x–1.74	0.947	0.053
	Pi5	50–80	17.78–33.33		y = 0.53x–8	0.980*	0.020
	Pi6	50–80	31.03–44.83		y = 0.45x+7.93	0.983*	0.017
Aqueous	Pi1	50–80	2.13–27.66		y = 0.77x–41.28	0.775	0.225
	Pi3	50–80	4.08–16.33		y = 0.35x–13.88	0.834	0.166
	Pi5	50–80	0.0–2.22		y = 0.09x–4.67	0.775	0.225
	Pi6	50–80	0.0–2.22		y =0.068x–3.78	0.894	0.106
Garlic/ Methanol	Pi1	5–30 40–50	2.56–41.03 100	31.5	y = 2.46x–18.15	0.947**	0.004
	Pi3	5–30 40–50	5.0–42.5 100	31.25	y = 2.24x–7.51	0.955**	0.003
	Pi5	5–30 40–50	4.88–46.34 100	30.5	y = 2.41x–15.13	0.952**	0.003
	Pi6	5–30 40–50	0.0–48.78 100	30.25	y = 2.52x–19.15	0.960**	0.002
Hexane	Pi1	50–100	0.0–7.41		y = 0.143x–6.68	0.938*	0.018
	Pi3	50–100	0.0–1.96		y = 0.048x–2.54	0.866	0.058
	Pi5	50–100	1.89–7.55		y = 0.066x–1.02	0.970**	0.006
	Pi6	50–100	0.0–6.0		y = 0.122x–7.49	0.894*	0.041
Aqueous	Pi1	50–100	1.82–3.64		y = 0.034x+0.66	0.707	0.182
	Pi3	50–100	0.0–1.96		y = 0.037x–1.325	0.707	0.182
	Pi5	50–100	0.0–3.85		y = 0.081–3.98	0.945*	0.015
	Pi6	50–100	1.89–7.55		y =0.077x–0.31	0.516	0.373
Sticky fleabane/ Methanol	Pi1	50–80	100	25			
	Pi3	50–80	100	25			
	Pi5	50 60–80	71.93 100	34.5	y = 0.842x+38.25	0.775	0.225
	Pi6	50 60–80	68.97 100	36	y = 0.93x+31.72	0.775	0.225

1	2	3	4	5	6	7	8
Hexane	Pi1	40–90	36.84–59.65		y = 0.46x+17.911	0.993**	0.001
	Pi3	40–90	16–56.00		y =0.751x–7.98	0.949*	0.014
	Pi5	40–90	36.84–59.65		y = 0.447x+20.34	0.961**	0.009
	Pi6	40–90	31.03–60.35		y =0.503x+16.09	0.911*	0.031
Harmal Methanol	Pi1	50–100	38.60–57.89		y = 0.322x+19.06	0.844	0.072
	Pi3	50–100	34–48.00		y = 0.259x+20.16	0.883*	0.047
	Pi5	50–100	38.60–45.61		y = 0.147x+29.59	0.892*	0.042
	Pi6	50–100	33.33–47.06		y = 0.321x+14.6	0.932*	0.021
Hexane	Pi1	50–100	13.64–25.00		y = 0.264x+0.307	0.889*	0.044
	Pi3	50–100	20.41–32.65		y = 0.234x + 10.3	0.879	0.051
	Pi5	50–100	20.41–34.69		y = 0.314x+3.64	0.965**	0.008
	Pi6	50–100	12–36.00		y = 0.478x–12.51	0.994**	0.001
Between fractions	Pi1	50–100	15.91–31.82		y = 0.310x+1.26	0.943*	0.016
	Pi3	50–100	34.69–42.86		y = 0.138x+28.02	0.894*	0.041
	Pi5	50–100	20.41–40.82		y = 0.483x–2.951	0.923*	0.025
	Pi6	50–100	10–46.00		y = 0.684x–20.14	0.897*	0.039
Nightshade fruit Methanol	Pi1	50–80	29.55–34.09		y = 0.136x+22.96	0.949	0.051
	Pi3	50–80	40.82–42.86		y = 0.061x+37.44	0.775	0.225
	Pi5	50–80	32.65–42.86		y = 0.367x+12.86	0.949	0.051
	Pi6	50–80	41.18–45.09		y = 0.118x+35.49	0.949	0.051
Hexane	Pi1	50–80	29.55–38.64		y = 4.546x+25	1.000**	0.000
	Pi3	50–80	36.74–42.86		y = 3.061x+33.33	0.982	0.121
	Pi5	50–80	34.69–40.82		y = 3.06x+31.97	0.982	0.121
	Pi6	50–80	35.29–50.98		y = 7.843x+26.79	0.990	0.091
Aqueous	Pi1	50–80	16.667–29.17		y = 0.479x–8.75	0.927	0.073
	Pi3	50–80	24.49–36.74		y = 0.449x+0.41	0.947	0.053
	Pi5	50–80	4.08–30.61		y = 0.857x–35.31	0.949	0.051
	Pi6	50–80	11.76–35.29		y = 0.804x–27.3	0.984*	0.016
Nightshade leaves Methanol	Pi1	50–90	13.64–31.82		y = 0.455x–11.4	0.978*	0.022
	Pi3	50–90	24.49–32.65		y = 0.181x+16.0	0.832	0.168
	Pi5	50–90	28.57–36.74		y = 0.181x+20.06	0.832	0.168
	Pi6	50–90	29.41–43.14		y = 0.359x+11.26	0.980*	0.020
Hexane	Pi1	50–80 90	35.42–50.00 100	80	y = 1.41x–45.595	0.884	0.116
	Pi3	50–80 90	44.89–55.10 100	73.5	y = 1.184x–24.10	0.865	0.135
	Pi5	50–90	36.73–46.94		y = 0.268x+24.43	0.868	0.132
	Pi6	50–90	41.18–50.98		y = 0.263x+28.46	0.929	0.071
Aqueous	Pi1	50–90	31.82–47.73		y = 0.331x+12.92	0.847	0.153
	Pi3	50–90	30.61–38.78		y = 0.216x+20.58	0.887	0.113
	Pi5	50–90	32.65–44.90		y = 0.367x+14.69	0.959*	0.041
	Pi6	50–90	40.0–44.0		y = 0.097x+34.46	0.944	0.056
Between fractions	Pi1	50–90	22.22–42.22		y = 0.476x+2.857	0.992**	0.008
	Pi3	50–90	30.60–38.78		y = 0.163x+22.86	0.849	0.151
	Pi5	50–90	32.65–48.97		y = 0.402x+11.14	0.996**	0.004
	Pi6	50–90	40.38–50.0		y = 0.242x+28.63	0.956*	0.044

[a] % of mycelial growth inhibition was determined from the mean diameter [mm] of control colony on non-amended medium and that tested on amended plate (zone of growth)
[b] concentration of plant fraction producing 50% fungal growth inhibition
[c] regression analysis for the relationship between the size of inhibition zone (mm) and the concentration [μg] of crude extract or fraction (Log-value) was calculated using Microsoft Excel 2003 and SPSS program version 10
[d] correlation coefficient values
[e] level of significance at the 0.05 and 0.01 levels (2-tailed). Values are means of ± SD of at least two independent experiments
* correlation is significant at the 0.05level (2-tailed)
** correlation is significant at the 0.01 level (2-tailed)

phal growth of isolates Pi.1 and Pi.3 at a concentration of 90 μg/ml with the IC_{50} values for these isolates 80 and 73.5 μg/ml, respectively. However, isolates Pi.5 and Pi.6 yielded fixed inhibition percentage values at concentrations of 80 and 90 μg/ml (Table 2). In contrast, none of the concentrations of harmal, sticky fleabane, garlic, fenugreek or nightshade fruit hexane fractions tested gave complete inhibition of fungal growth. However, highly reduced non-conidiating growth was observed with the sticky fleabane leaf fraction. Concerning the effect of aqueous fractions on fungal growth inhibition, the data presented in table 2 indicate that the cinnamon fraction was more effective against isolates Pi.1 and Pi.5 (but not Pi.3 and Pi.6) where complete inhibition of fungal growth was obtained at concentrations of 70 and 80 μg/ml, and this was reflected by IC_{50} values of 61.25 and 58.5 μg/ml, respectively. However, no complete inhibition of any of the isolates was observed by the same fraction or the layer between fractions from the remaining plants tested.

Sensitivity of *P. italicum* isolates infecting lemon fruit to crude plant extracts

The results presented in table 3 indicate that the lemon fruit treated with plant extracts were more susceptible to fungal infections than orange fruit. However, extracts of fenugreek seeds, harmal seeds and sticky fleabane leaves were the most effective in terms of growth restriction of isolate Pi.1 infecting lemon fruit, where complete inhibition of fungal growth was achieved at a concentration within the range of 390 to 520 μg/ml. However, the growth of isolate Pi.3 on lemon fruit was highly susceptible to nightshade fruit and sticky fleabane extracts, where complete inhibition of fungal growth was obtained at all concentrations tested i.e. within the range of 130 to 520 μg/ml (Fig. 1a). Furthermore, nightshade fruit (Fig. 1b) and fenugreek extracts (Fig. 1c) were the most effective against the growth of fungal isolate Pi.5 (which infects lemon fruit), with complete inhibition within the concentration range 130 to 520 μg/ml.

Table 3. Sensitivity of *P. italicum* isolates infecting lemon fruits to different concentrations of plant extracts (*in vivo*)

Source of plant extracts	Conc.range [μg])[a]	Mean sizeof inhibition zone [mm]	IC_{50}	MIC	Corr. Value (r)[b] Sig[c]	Regression equation[d]	Fungal isolate
Nightshade fruits	130–520	18±1.32–35±0.92			0.8820.118	y = 28.40x–44.85	Pi.1
	100 130–520	34±0.98C.I[e]	107	130			Pi.3
	100 130–520	53±1.33100	94	130			Pi.5
Nightshade leaves	130–520	22±2.12–37±2.35			0.9780.022	y = 23.29x–26.79	Pi.1
	130–520	25±1.54–43±2.14			0.8870.113	y = 27.33x–35.23	Pi.3
	130–520	14±1.11–34±2.15			0.984*0.016	y = 31.48x–51.92	Pi.5
Cinnamon	130–520	8±1.21–34±2.32			0.9210.079	y = 40.78x–81.32	Pi.1
	130–520	17±0.89–27±1.35			0.993**0.007	y = 16.17x–17.53	Pi.3
	130–520	11±1.11–27±2.14			0.9450.055	y = 27.75x–49.26	Pi.5
Fenugreek	130–260 390–520	27±1.32–29±1.14C.I	288.40	389.11	0.7950.415		Pi.1
	130–520	23±2.11–38±1.54			0.8930.107	y = 22.07x–25.80	Pi.3
	100 130–520	43±2.22C.I	104	130			Pi.5
Harmal	130–260 390–520	27±2.25–29±2.22C.I	288.4	398.11	0.7950.415	y = 137.36x–273.9	Pi.1
	130–390 520	23±1.17–37±1.36C.I	407.380	512.86	0.9980.044	y = 29.103x–38.73	Pi.3
	130–520	17±0.89–36±1.35			0.972*0.028	y = 29.40x–46.08	Pi.5
Garlic	130–520	20±1.35–32±2.33			0.9440.056	y = 17.63x–17.37	Pi.1
	130–520	20±0.96–29±0.87			0.980*0.020	y = 14.48x–10.11	Pi.3
	130–260 390–520	22±0.35–36±2.14C.I	281.84	398.11	0.8750.322	y = 151.07x–305.8	Pi.5
Sticky fleabane	130–390 520	22±1.32–33±0.96C.I	416.87	524.81	0.7500.250	y = 103.85x–209.5	Pi.1
	100 130–520	57±0.87C.I	87	130			Pi.3
	130–520	11±0.94–36±1.15			0.8870.113	y = 38.69x–74.42	Pi.5

[a] a range of concentrations (130, 260, 390 & 520 μg/ml) of extracts was used against tested fungal isolates
[b] correlation coefficient value
[c] level of significance at the 0.05 and 0.01 levels (2-tailed)
[d] regression analysis for the relationship between size of inhibition zone (mm) and the concentration [μg] of plant extract (Log-value). Values are means of two replicates and each experiment was repeated at least twice
[e] C.I denotes complete fungal growth inhibition
* correlation is significant at the 0.05 level (2-tailed)
** correlation is significant at the 0.01 level (2-tailed)

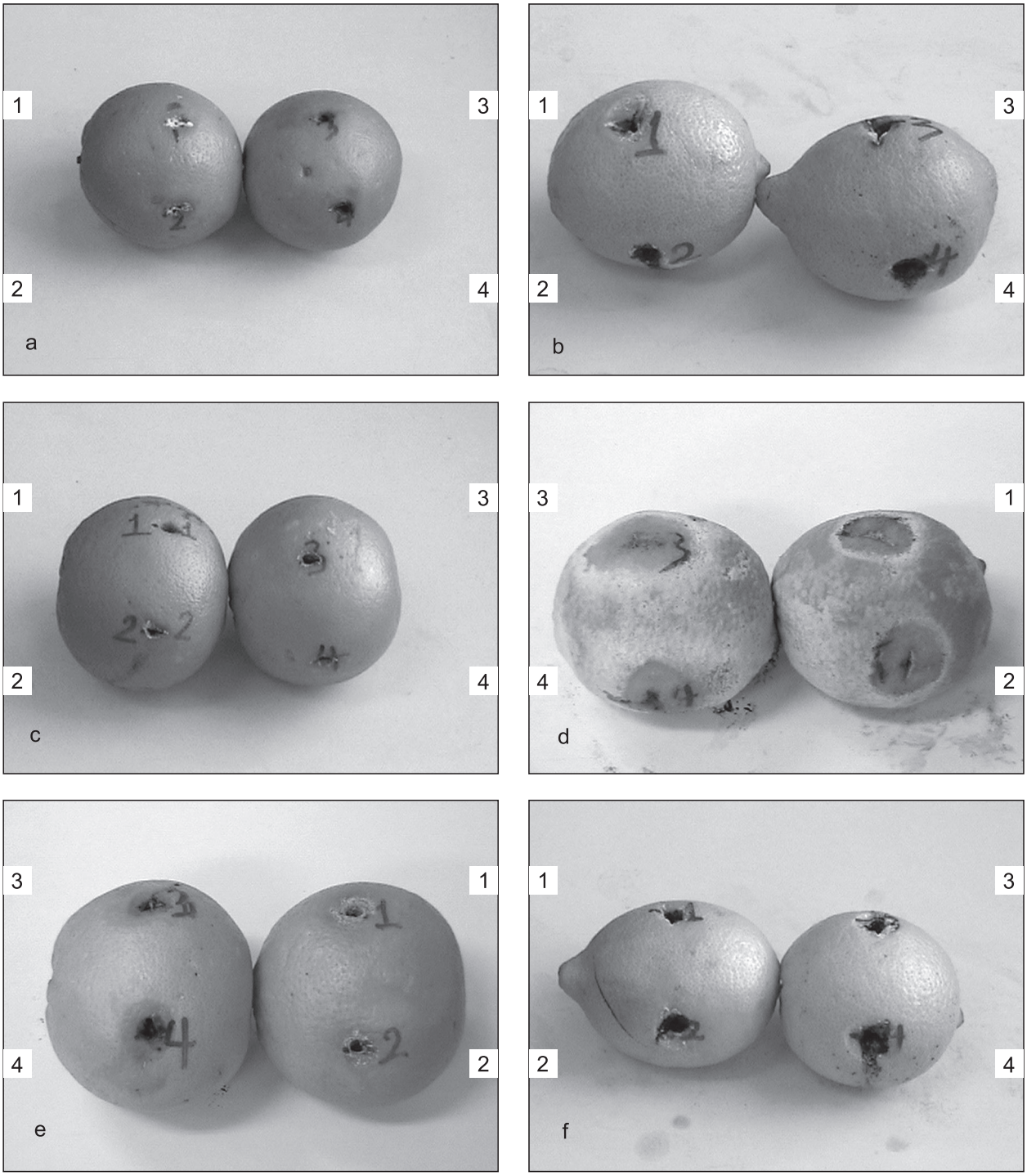

Fig. 1. *In vivo* study for the effect of different concentrations : (1), reflects 130 μg/ml, (2), 260 μg/ml, (3), 390 μg/ml, and (4), 520 μg/ml of plant extracts on growth of *P. italicum* isolates infecting citrus (orange and lemon) fruits. Panel a: represents the effect of sticky fleabane extract on growth of isolate Pi5. Panel b: represents the effect of sticky fleabane extract on growth of isolate Pi.3. Panel c: represents the effect of cinnamon extract on growth of isolate pi.3. Panel d: represents the effect of nightshade fruits extract on growth of isolate Pi1. Panel e: represents the effect of garlic extract on growth of isolate pi.3. Panel f: represents the effect of fenugreek extract on growth of isolate pi.5.

Sensitivity of *P. italicum* isolates infecting orange fruit to crude plant extracts

Orange fruit treated with the plant extracts were observed to be more tolerant to fungal infections than lemon fruit (Table 4). Complete growth inhibition of isolate Pi.1 infecting orange fruit was achieved at a concentration of 130 μg/ml when the fruit was treated with extracts of nightshade fruit, nightshade leaves, fenugreek seeds and sticky fleabane leaves. However, such inhibition of Pi 1 growth was achieved at a concentration of 390 μg/ml of cinnamon bark and garlic cloves extracts, whereas harmal seed extract resulted in growth inhibition at 260 μg/ml. In addition, the growth of isolate Pi.3 was completely inhibited on orange fruit when the fruit was treated with 130 μg/ml of the following extracts: nightshade leaves, cinnamon bark (Fig. 1d), fenugreek seeds and sticky flea-

bane leaves. However, 390 µg/ml of garlic cloves (Fig. 1e) or harmal seed extract also resulted in complete inhibition of isolate Pi.3 on orange fruit. Moreover, the growth of isolate Pi.5 infecting orange fruit was completely inhibited with 520 µg/ml of the following plants extracts: nightshade fruit, fenugreek and harmal seeds, whereas such inhibition was obtained at a concentration of 390 µg/ml of cinnamon bark and sticky fleabane extracts (Fig. 1f).

Table 4. Sensitivity of *P. italicum* isolates infecting orange fruits to different concentrations of plant extracts (*in vivo*)

Source of plant extracts	Conc. range [µg][a]	Mean size of inhibition zone [mm]	IC_{50}	MIC	Corr. Value (r)[b] Sig[c]	Regression equation[d]	Fungal isolate
Nightshade fruits	100 130–520	45±3.12 C.I	103	130			Pi.1
	130–520	17±1.25–31±2.24	83.8	389.05	0.966* 0.034	y = 24.34x–33.37	Pi.3
	130–390 520	5±1.11–13±1.24 C.I	446.68	512.86	0.992 0.082	y =17.148x–31.03	Pi.5
Nightshade leaves	100 130–520	48±2.36 C.I	101	130			Pi.1
	100 130–520	43±2.33 C.I	104	130			Pi.3
	130–520	3±0.78–6±0.68			0.819 0.181	y = 4.417x–6.865	Pi.5
Cinnamon	130–260 390–520	19±1.12–26±1.34 C.I	288.40	380.19	0.827 0.380	y = 154.15x –317.5	Pi.1
	100 130–520	58±1.25 C.I	86	130			Pi.3
	130–260 260 390–520	12±1.11–17±1.26 C.I	309.03	398.11	0.811 0.398	y = 166.53x–352.2	Pi.5
Fenugreek	100 130–520	52±1.25 C.I	95	130			Pi.1
	100 130–520	66±1.27 C.I	76	130			Pi.3
	130–390 520	5±0.85–11±1.02 C.I	457.09	512.86	0.699 0.301	y = 123.23x–272.3	Pi.5
Harmal	130 260–520	24±1.24 C.I	165.96	263.03	0.932 0.237	y = 169.79x–328.2	Pi.1
	130–260 390–520	2±0.42–3±0.62 C.I	316.23	389.05	0.786 0.424	y = 183.79x–401.1	Pi.3
	130–390 520	2±0.35–5±0.47 C.I	457.09	514.81	0.682 0.318	y = 124.91x–279.2	Pi.5
Garlic	130260 390–520	17±1.23–22±2.21 C.I	301.99	380.19	0.813 0.396	y = 157.17x–326.6	Pi.1
	130–260 390–520	2±0.53–3±0.47 C.I	316.23	389.05	0.786 0.486	y = 183.79x–401.1	Pi.3
	130–520	6±0.65–8±0.65			0.864 0.136	y = 3.155x–1.011	Pi.5
Sticky fleabane	100 130–520	49±1.25 C.I	100	130			Pi.1
	100 130–520	55±2.23 C.I	91	130			Pi.3
	130–260 390–520	12±1.12–23±1.41 C.I	301.99	398.11	0.847 0.357	y = 163.7x–355.32	Pi.5

[a] range of concentrations (130, 260, 390 & 520 µg/ml) of extracts was used against tested fungal isolates
[b] correlation coefficient value
[c] level of significance at the 0.05 and 0.01 levels (2-tailed
[d] Regression analysis for the relationship between size of inhibition zone [mm] and the concentration [µg] of plant extract (Log-value). Values are means of two replicates and each experiment was repeated at least twice
[e] C.I denotes complete fungal growth inhibition
* correlation is significant at the 0.05 level (2-tailed).
** correlation is significant at the 0.01 level (2-tailed)

DISCUSSION

Our results demonstrate that crude extracts as well as liquid fractions of sticky fleabane, cinnamon, garlic, harmal, fenugreek and nightshade plants possess effective *in vitro* and *in vivo* antifungal activity against *P. italicum* isolates. Moreover, extracts of sticky fleabane, harmal and fenugreek generated complete growth inhibition of fungal isolates infecting both lemon and orange fruit. In addition, extracts of cinnamon and nightshade leaves caused complete inhibition of isolates infecting orange fruit, while garlic extract showed such inhibition to isolates that infect lemon fruit.

The present study also showed that cinnamon extract possesses high activity against the fungal isolates of *P. italicum*, where the crude extract as well as methanolic, hexane and aqueous fractions gave complete inhibition of fungal growth. The antifungal activity of cinnamon may be related to active antimicrobial agents present in the extract and fractions including mainly cinnamaldehyde and eugenol as well as cinamic acid, flavonoids, alkaloids, tannins, anthraquinones, and phenolic compounds. This explanation is in agreement with the findings of Rojas and co-workers (1992) who identified such components as active antifungal agents. Furthermore, eugenol and cinnamaldehyde have been consistently reported by several research groups to be the main components of cinnamon exhibiting high fungitoxic activity (Gulab *et al.* 2005). In addition, cinnamaldehyde has been considered as a specific inhibitor of fungal cell wall synthesizing enzymes including ß-(1,3)-glucan synthase, a participant in biosynthesis of chitin and ß-glucans, the major structural components of the fungal cell wall (Cowan 1999). The results indicate that the crude extract and methanolic fraction of sticky fleabane possess high efficacy against *P. italicum* isolates. These findings agree with those of Wang *et al.* (2004) who suggested that *Inula* extract has broad spectrum activity against several fungal species infecting various crop plants. In addition, results presented by Cohen *et al.* (2002) indicated that extracts of *I. viscosa* prepared using organic solvents showed *in vitro* antifungal activity as well as an inhibitory effect on chitin biosynthesis. Such strong inhibitory activity may be related to a high content of flavonoids, phenolic compounds and anthraquinones present in methanolic and aqueous fractions of *I. viscosa*. These observations broadly agree with those proposed previously by other researchers (Shtayeh and Abu Gheleib 1999; Cohen *et al.* 2002). In contrast, our results disagreed with the findings of Muller-Riebau and co-workers (1997) who found small amounts of antifungal essential oil or phenolics. They concluded that the plant has no economic value for producing antifungal preparations.

Our results indicate that harmal extract was the second most effective preparation against *P. italicum* growth *in vitro*. In contrast none of its fractions provided complete inhibition of fungal growth. A strong inhibitory activity of the crude extract may be related to the high content of alkaloids (harmine, harmaline and tetrahydroharmine) and phenolic compounds. Phenolic compounds might alter fungal cell permeability and thus permit loss of macromolecules (Rasooli 2004). The exact mode of action of phenols has not been determined, but they may inactivate essential enzymes, react with cell membrane proteins or even disrupt function of the genetic material (Telezhenetskaya and Dyakonov 2004).

Garlic extract was not effective in terms of completely inhibiting the growth of *P. italicum* isolates as compared to the activity of the methanolic fraction that caused complete inhibition (*in vitro*). However, garlic extract was more effective against fungal isolates infecting lemon fruit. Surprisingly, a remarkable improvement in its activity was observed when extraction was performed in presence of a small volume of olive oil which slows down the breakdown process of allicin (Marino *et al.* 2001; Obagwu and Korten 2003). The strong antifungal activity of garlic is related to allicin (dially thiosulfinate: the main biologically active component) which reflects inhibitory activity against enzymes essential for pathogen infection (Marino *et al.* 1999).

Similarly, ajoene, which is an allicin derivative, also exhibited strong inhibitory activity against several fungal species including *Aspergillus niger* and *Candida albicans* (Gurib-Fakim *et al.* 2005). Furthermore, Yoshida *et al.* (1987) reported that ajoene was superior to allicin in the efficacy of fungal growth inhibition since it disrupts the fungal cell wall. Moreover, morphological changes such as disappearance of surface ornaments, thickening of cell wall and destruction of cell organelles may lead to the conclusion that ajoene has acted on the cell wall (Masperi *et al.* 1984; Yoshida *et al.* 1987). These findings agreed also with those of Soylu *et al.* (2005) who stated that the aqueous extract of garlic causes morphological alterations in the hyphae of several fungal species including *Pythium ultimum*, *Rhizoctonia solani*, *Colletotrichum lindemuthianum* and *Fusarium solani*. Such hyphae appeared smaller in diameter after treatment with garlic extract.

A crude extract of fenugreek displayed the third most effective antifungal activity however complete inhibition of fungal growth was not observed. The fenugreek plant lacks flavonoids but is rich in alkaloids (in both the methanolic and aqueous fractions)and phenolic compounds (aqueous layer). However, the fractionation process might have weakened their cumulative activities against fungal growth. It has been reported that the methanolic extract of fenugreek was highly specific for dermatophytes (Shtayeh and Abu Ghdieb 1999; Olli and Kirti 2006). In addition, fenugreek extract showed antifungal activity against other fungal species. For instance, Olli and Kirti (2006) reported that the Tfg d1 protein (defensins of fenugreek) possessing eight cysteine residues plays a vital role in inhibiting the spread of *R. solani* causing crescent growth inhibition. A reduced *in vitro* but not the *in vivo* antifungal activity observed with the crude extract and fractions of nightshade leaves and fruit could be related to the presence of many steroid alkaloids (solamargine, solasomine, solamine and saponin) in the layer between fractions. These steroid alkaloids have been shown to display antifungal activity against eleven agronomically important fungi including *Aspergillus* spp., *Rhizopus* spp., *Fusarium* spp. and others (AL-Fatimi *et al.* 2007).

Several studies have been conducted to shed light on the mechanism(s) of action of several active components plant extracts including essential oils (Chang *et al.* 2001). However, such mechanisms are still unclear although some studies suggested that these compounds penetrate inside the cell, where they interfere with cellular metabolism (Marino *et al.* 2001). Other studies suggested that plant extracts can disrupt the plasma membrane of fungal cell and react with active sites of membrane proteins or enzymes or even act as a proton carrier and consequently deplete the adenosine triphosphate pool (Chang *et al.* 2001; Ultee *et al.* 2002). A comparative study with results from previous work which involved the use of the same plants materials against *P. digitatum* isolates revealed that the plant materials were more effective in controlling the growth of *P. digitatum* isolates (Kanan and AL-Najar 2008). In addition complete inhibition of *P. digitatum* isolates was obtained with the crude extracts of nightshade fruit, cinnamon bark and fenugreek seeds at MIC values within the range of 130–520 μg/ml. Conversely, none of the extracts produced complete inhibition of *P. italicum* isolates (Kanan and AL-Najar 2008). Furthermore, methanolic fractions of all the plants tested (except fenugreek) caused complete inhibition of *P. digitatum* isolates, whereas with *P. italicum* isolates only cinnamon, garlic and sticky fleabane methanolic fractions resulted in complete inhibition. Moreover, the hexane fraction of all plants tested (except fenugreek and garlic) resulted in complete inhibition of *P. digitatum* whereas with *P. italicum* isolates only cinnamon and nightshade leaves produced complete inhibition (Kanan and AL-Najar 2008). Notwithstanding, the findings of both research studies revealed that fenugreek crude extract as well as its fractions were ineffective in controlling the growth of fungal isolates of both species. Finally, the garlic methanolic fraction was the only fraction that caused complete inhibition of isolates from both tested *Penicillium* species (Kanan and AL-Najar 2008).

ACKNOWLEDGEMENTS

The Authors would like to thank Mu'tah University, Jordan for providing the requirements and a suitable environment for this research work. Thanks are also due to Dr. Saleh Al-Quran for his help in classifying the tested plant species.

REFERENCES

Abad M.J., Ansuategui M., Bermejo P. 2007. Active antifungal substances from natural sources. Arkivoc. (VII): 116–145.

Abu Zarga M.H., Hamed E.M., Sabri S.S., Voelter W., Zeller K.P. 1998. New sesquiterpenoids from the Jordanian medicinal plant *Inula viscosa*. J. Nat. Prod. 61(6): 798–800.

AL-Fatimi M., Wurster M., Schroder G., Lindequist U. 2007. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J. Ethnopharmacol. 111(3): 657–666.

Ambrozin A.R.P., Vieira P.C., Fernandes J.B., Da Silva M.F.G.F., Albuquerque S. 2004. Trypanocidal activity of *Meliaceae* and *Rutaceae* plant extracts. Mem. Inst. Oswaldo. Cruz. 99 (2): 227–231.

Cafarchia C., De Laurentis N., Milillo M.A., Losacco V., Puccini V. 2002. Antifungal activity of essential oils from leaves and flowers of *Inula viscosa* (Asteraceae) by Apulian region. Parassitologia 44 (3–4): 153–156.

Chang S.T., Chen P.F., Chang S.C. 2001. Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*. J. Ethnopharmacol. 77: 123–127.

Cohen Y., Baider A., Ben-Daniel B.H., Ben-Daniel Y. 2002. Fungicidal preparations from *Inula viscosa*. Plant Protect. Sci. 38: 629–630.

Cohen Y., Wang W., Ben-Daniel B.H., Ben-Daniel Y. 2006. Extracts of *Inula viscosa* control downy mildew of grapes caused by *Plasmopara viticola*. Phytopathology 96 (4): 417–424.

Cove D.J. 1966. The induction and repression of nitrate reductase in the fungus *Aspergillus nidulans*. Biochem. Biophys. Acta 113: 51–56.

Cowan M.M. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12: 564–582.

Delaquis P.J., Stanich K., Girard B., Mazza G. 2002. Antimicrobial of individual and mixed fraction of dill, cilantro, coriander and eucalyptus essential oils. Int. J. Food. Microbiol. 74: 101–109.

Elsom G.K. 2000. An antibacterial assay of aqueous extract of garlic against anaerobic/microaerophilic and aerobic bacteria. Microb. Ecol. Health. Dis. 12: 81–84.

Gulab N.J., Onker D.D., Carolina M.J., Vania M.M.V. 2005. Identification of the major fungitoxic component of Cinnamon bark oil. Fitopatol. Bras. 30 (4): 404–408.

Gurib-Fakim A., Subratty H., Narod F., Govinden-Soulange J., Mahomoodally F. 2005. Biological activity from indigenous medicinal plants of Mauritius. Pure. Appl. Chem . 77: 41–51.

Hammer K.A., Carson C.F., Riley T.V. 1999. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86: 985–990.

Kanan G.J., AL-Najar R.A. 2008. In vitro antifungal activities of various plant crude extracts and fractions against citrus post-harvest disease agent *Penicillium digitatum*. Jor. J. Biol. Sci. 1 (3): 89–99.

Marino M., Bersani C., Comi G. 1999. Antimicrobial activity of the essential oils of *Thymus vulgaris L.* Measured using a bioimpedometric method. J. Food. Prod. 62 (9): 1017–1023.

Marino M., Bersani C., Comi G. 2001. Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. Int. J. Food. Microbiol. 67: 187–195.

Masperi P.G., Dall'olio A., Calefano G.L.V. 1984. Autophagic vacuole development in *Trichophyton mentagrophytes* exposed in vitro to miconazole. Sabouraudia 22: 27–35.

Muller Riebau F.J., Berger B., Yegen O. 1997. Chemical composition and fungitoxic properties to phytopathogenic fungi essential oils of selected aromatic plants growing wild in Turkey. J. Agric. Food. Chem. 43: 2262–2266.

Muto M., Mulabagal V., Huang H.C., Takahashi H., Tsay H.S., Huang J.W. 2006. Toxicity of black nightshade (*Solanum nigrum*) extracts on *Alternaria brassicicola*, causal agent of black leaf spot of Chinese cabbage (*Brassica pekinensis*). J. Phytopath. 154 (1): 45–50.

Ndukwe I.G., Habila J.D., Bello I.A., Adeleye E.O. 2006. Phytochemical analysis and antimicrobial screening of crude extracts from the leaves, stem bark and root bark of *Ekebergia senegalensis A. Juss*. Afr. J. Biotech. 5 (19): 1792–1794.

Nwachukwu E.O., Umechuruba C.I. 2001. Antifungal activities of some leaf extracts on seedborne fungi of Africa Yam bean seeds, seed germination and seedling emergence. J. Appl. Sci. Environ. Mgt. 5 (1): 29–32.

Obagwu J., Korsten L. 2003. Control of citrus green and blue molds with garlic extracts. Eur. J. Plant. Path. 109: 221–225.

Olli S., Kirti P.B. 2006. Cloning, characterization and antifungal activity of Defensin Tfgd1 from *Trigonella foenum-graecum* L. J. Biochem. Mole. Biol. 39: 278–283.

Prusky D., McEvoy J.L., Saftner R., Conway W.S., Jones R. 2004. Relationship between host acidification and virulence of *Penicillium* spp. on apple and citrus fruit. Bioch. Cell. Biol. 94 (1): 44–51.

Rasooli I., Abyaneh M.R. 2004. Inhibitory effect of thyme oils on growth and aflatoxin production by *Apergillus parasiticus*. Food. Cont. 15: 479–483.

Rojas A., Hernandez L., Pereda-Miranda R., Mata R. 1992. Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. J. Ethnopharmacol. 35: 275–283.

Shtayeh M.S., Abu Ghdeib S.I. 1999. Antifungal activity of plant extract against dermatophytes. Mycoses. 42 (11–12): 665–672.

Souza-Fagundes E.M., Queiroz A.B.R., Filho O.A.M., Gazzinelli G., Corrâ-Oliveira R., Alves T.M.A., Zani C.L. 2002. Screening and fractionation of plant extracts with antiproliferative activity on human peripheral blood mononuclear cells. Mem. Inst. Oswaldo. Cruz. 97 (8): 1207–1212.

Soylu E.M., Tok F.M., Soylu S., Kaya A.D., Evrendilek G.A. 2005. Antifungal activities of essential oils on post harvest disease agent *Penicillium digitatum*. Pakistan. J. Biol. Sci. 8 (1): 25–29.

Telezhenetskaya M.V., D'yakonov A.L. 2004. Alkaloids of *Peganum harmala*. Unusual reaction of peganine and vasicinone. Chem. Nat. Comp. 27: 471–474.

Ultee A., Bennik M.H.J., Moezelaar R. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-born pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 68: 1561–1568.

Wang W.Q., Ben-Daniel B.H., Cohen Y. 2004. Extracts of *Inula viscosa* control downy milew caused by *Plasmopara viticola* in grape-vines. Phytoparasitica 32: 208–211.

Yoshida S., Kasuga S., Hayashi N., Ushiroguchi T., Matsuura H., Nakagawa S. 1987. Antifungal activity of Ajoene derived from garlic. Appl. Environ. Microbiol. 53: 615–617.

Zhang H.Y., Fu C.X., Zheng X.D., He D., Shan L.J., Zhan X. 2004. Effect of *Cryptococcus laurentii* (Kufferath) Skinner in combination with sodium bicarbonate on biocontrol of post harvest green mold decay of citrus fruit. Bot. Bull. Acad. Sinica 45: 159–164.

Zhou X., He X., Wang G., Gao H., Zhou G., Ye W. 2006. Steroidal saponins from *Solanum nigrum*. J. Nat. Prod. 69 (8): 1158–1163.

Zhu J.W., Xie Q.Y., Li H.Y. 2006. Occurrence of imazalil-resistant biotype of *Penicillium digitatum* in China and the resistant molecular mechanism. J. Zhejiang University Sci. 7 (11): 362–365.

POLISH SUMMARY

AKTYWNOŚĆ *IN VITRO* I *IN VIVO* WYBRANYCH WYCIĄGÓW ROŚLINNYCH I ICH FRAKCJI PRZECIWKO *PENICILLIUM ITALICUM*

Oceniano działania (*in vitro* i *in vivo*) siedmiu rodzajów wyciągów roślinnych i ich płynnych frakcji przeciwko izolatom *Penicillium italicum.* Badania *in vitro* wykazały, że surowy wyciąg z liści *Inula viscosa* lub kory cynamonu w stężeniu 520 μg/ml, wykazywał maksymalne właściwości inhibicyjne przeciwko badanym izolatom grzyba, które wynosiły odpowiednio 54% i 43%. Każdy z wyciągów użyty w stężeniu 130 μg/ml powodował całkowitą inhibicję wzrostu izolatów grzyba Pi.1 i Pi.3 infekujących owoce pomarańczy (wyjątkiem był *Peganum harmala* i czosnek, dla których konieczne było stężenie 390 μg/ml). W przypadku wyciągów z owoców *Solanum nigrum*, *Trigonella foenum-graecum* i *Inula viscosa* stężenie 130 μg/ml wywołało całkowitą inhibicję wzrostu izolatów Pi.3 i Pi.5, infekujących owoce cytryny, a do inhibicji wzrostu izolatu Pi.1 potrzebne było stężenie 390 μg/ml. Frakcje metanolowe wyciągów z cynamonu, czosnku i *I. viscosa* inhibitowały całkowicie wzrost grzybów. Stwierdzone wartości IC_{50} dla wyżej wspomnianych frakcji wynosiły odpowiednio: 11,2–24; 30,25–31,50; 25,0–36,0 μg/ml. Stężenie 20 μg/ml heksanowej frakcji cynamonu inhibitowało wzrost testowych izolatów, a stwierdzone wartości IC_{50} przeciwko izolatom Pi.1, Pi.3, Pi.5 i Pi.6 wynosiły odpowiednio 13, 13,75, 14 i 13 μg/ml. Heksanowi frakcja *S. nigrum* całkowicie inhibitowała wzrost izolatów Pi.1 i Pi.3 (wartości IC_{50} wynosiły odpowiednio 80 i 37,5 μg/ml). Wodna frakcja cynamonu całkowicie inhibitowała wzrost izolatów Pi.1 i Pi.5 (wartości IC_{50} wynosiły odpowiednio 61,25 i 58,5 μg/ml).