Ann Microbiol (2012) 62:1021–1029
DOI 10.1007/s13213-011-0342-2

ORIGINAL ARTICLE

Antifungal activity of some plant extracts against sugar beet damping-off caused by *Sclerotium rolfsii*

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Received: 18 March 2011 / Accepted: 2 August 2011 / Published online: 2 September 2011

Abstract In an attempt to search for natural pesticides, crude extracts of seven plant species (*Bauhinia purpurea*, *Caesalpinia gilliesii*, *Cassia fistula*, *Cassia senna*, *Chrysanthemum frutescens*, *Euonymus japonicus* and *Thespesia populnea* var. *acutiloba*) were evaluated against *Sclerotium rolfsii*, the causative fungus of damping-off, under laboratory and greenhouse conditions. Gas chromatography-mass spectrometry analysis was performed to identify possible biologically active components (tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, phytol, linalool, 1,8 cineole and 9, 12, 15 octadecanoic acid) from the plant extracts most effective against *S. rolfsii*. Laboratory experiments indicated that leaf extracts of *T. populnea* var. *acutiloba* and *Chrysanthemum frutescens* were most effective against *S. rolfsii*. Greenhouse experiments confirmed that *T. populnea* var. *acutiloba* and *Chrysanthemum frutescens* extracts were most effective against the damping-off pathogen, either by coating or soaking of sugar beet seeds. None of the extracts tested produced phytotoxic effects on sugar beet leaves, even at the highest concentration applied. The most effective plant extracts showed low toxicity in rats relative to controls with respect to histological tests. The extracts assayed represent a potentially safe control method for damping-off disease in sugar beet.

Keywords Analysis · Extract · Pathogen · Sugar beet

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Introduction

Sugar beet (*Beta vulgaris* L., Chenopodiaceae) is one of the most important crops grown in temperate regions for sugar production. In Egypt, it is ranked as the second crop after sugar cane for sugar production (Eweis et al. 2006). Due to the daily demand for sugar, there is a need to increase the production of the sugar beet crop. Sugar beet is attacked by several pathogens and root-rot diseases, among which are those caused by *Rhizoctonia solani* and *S. rolfsii* (El-Abyad et al. 1997).

Scleritium rolfsii is a soil-borne fungus that causes damping-off disease on a wide range of agricultural and horticultural crops, as well as weeds and forest trees. The fungus is distributed in tropical and subtropical regions. It is quite common in the Southern United States, as well as central and South America. It has also been reported in Africa, Asia, Australia and parts of Europe (Aycock 1966). *S. rolfsii* is considered the most frequent, common and serious pathogen that attacks sugar beet roots, causing economic losses in the crop (Gouda 2001; Cramer et al. 2003). The pathogen is difficult to control because of the production of hardy resistant survival structures called sclerotia (Elad 1995). *S. rolfsii* is thought to have caused serious crop losses over many centuries (Punja 1985).

The control of plant diseases has for many years been based on the application of chemical pesticides. However, these pesticides are not effective for long-term use due to

concerns of expense, exposure risks, residues and other health and environmental hazards. Moreover, the potential for the development of resistance towards synthetic fungicides in pathogenic fungi is of great concern. Therefore, there is a great incentive to develop alternative safe, effective, and environmentally friendly fungicides (Mdee et al. 2009). Recent efforts have focused on the development of long-lasting and environmentally safe methods for the control of plant diseases. The use of plant products has been shown to be eco-friendly and effective against many plant pathogens (Latha et al. 2009). Presently, a renewed search for natural products with novel uses, particularly for pest management, is required. Most of these substances have been tested against pests in order to establish new control practices with low mammalian toxicity and low persistence in the environment. Therefore, research should focus not only on the efficacy of botanical extracts against target pests, but also their safety with regard to human health. An assessment of enzymatic activity in the blood is generally a more sensitive measure of a compound's toxicity than assessment of histopathological changes; the latter can be assessed within a shorter time period but may be less sensitive. Nevertheless, observation of tissue alterations is considered to have a confirmatory and supporting diagnostic role for detecting certain blood abnormalities, and so may have potential relevance as a preliminary test for the toxicity of botanical extracts (Cornelius et al. 1959). Most of the selected extracts in this study were confirmed by their natural origin and safety as human medicines (Park et al. 2005; Panda and Kar 1999). Moreover, no evidence of teratogenic or genotoxic activity has been detected resulting from the use of these plant extracts for pest control (Mengs et al. 2004; Mitchell et al. 2006). This is despite the fact that these plants are available in high amount in Egypt.

The objectives of the present study were to investigate the efficacy of newly used plant extracts on the growth activities of *S. rolfsii* under laboratory and greenhouse conditions. Other objectives were to identify by gas chromatography-mass spectrometry (GC-MS) analysis the biologically active compounds of the most effective plant extracts, and finally to evaluate the toxicity of the most effective plant extracts on rats using histology tests.

Materials and methods

Source of assay materials

The leaves of seven medicinal plant species (*Bauhinia purpurea*, *Caesalpinia gilliesii*, *Cassia fistula*, *Cassia senna*, *Chrysanthemum frutescens*, *Euonymus japonicus* and *Thespesia populnea* var. *acutiloba*) were collected from local nurseries in Kafr El-Sheikh, Monofia, Gharbia and Alexandria Governorates, Egypt. The leaves were oven-dried for 24 h at 70°C, and finely powdered using a blender. Each sample (25 g) was extracted twice with 300 ml methanol at room temperature for 2 days. The extracts were filtered through filter paper (no. 15, Whatman, Piscataway, NJ) and the combined filtrates from the twice-extracted leaves were concentrated to dryness by rotary evaporation at 40°C. The yield of each methanolic extract is given in Table 1.

The *S. rolfsii* isolate was obtained as a culture slant from the Plant Pathology Research Institute, Giza, Egypt. Glass bottles of 500 ml capacity, containing 100 g barley grains and 100 ml water, were autoclaved for 30 min at 1.5 atm, then inoculated with 7-day-old fungal culture and incubated at 28±1°C for 15 days. The culture in the glass bottles was used to inoculate soil in greenhouse experiments.

Synthetic fungicide

The synthetic fungicide tested in this study was thiram 37.5%+carboxin 37.5% and 25% Proprietary surfactants with a trade name of vitavax 75% WP, produced by Kafr-El-Zayat Co. (Kafr-El-Zayat, Egypt). This fungicide was applied at its recommended field rate of 2 g/kg seeds. In Egypt, this fungicide is highly recommended for the control of damping-off disease in sugar beet.

Screening of plant extracts against *S. rolfsii* under laboratory conditions

The seven extracts and thiram+carobxin were tested for their efficacy against *S. rolfsii* in a completely randomized design. The efficacy of the plant extracts and fungicide was determined as percent of inhibition of the growth of the selected fungus relative to the control treatment. Four concentrations for each plant extract (50, 100, 150 and 200 ppm) and four concentrations for the fungicide (1, 10, 25 and 50 ppm) were used. The required concentrations for plant extracts and fungicide were obtained by adding the appropriate amount of stock solution used to 60 ml portions of auto-calved potato dextrose agar (PDA) cooled to about 45°C. Four 9-cm-diameter glass Petri dishes were used as a replicate for each concentration of each treatment, including controls. Control treatment was carried out without adding fungicide or plant extracts. Each dish was inoculated in the center with a disk (5-mm diameter) bearing the mycelial growth from 5-day-old cultures of *S. rolfsii*. The dishes were sealed with Parafilm to avoid evaporation of volatile compounds. The dishes were incubated at 28°C until the controls achieved full growth, with mycelium reaching the edge of the plates. The inhibition percentage of radial growth of *S. rolfsii* was calculated using the

Table 1 List of plant species used for methanolic extraction and their yield

Family name	Scientific name	English name	Yield (%)[a]
Fabaceae	*Cassia senna*	Senna plant	10.4
Fabaceae	*Caesalpinia gilliesii*	Bird of paradise	10.6
Malvaceae	*Thespesia populnea* var. *acutiloba*	Wild tulip-tree	8.4
Asteraceae	*Chrysanthemum frutescens*	Marguerite daisy	15.2
Celastraceae	*Euonymus japonicus*	Winged spindle	11.2
Fabaceae	*Bauhinia purpurea*	Purple camel's foot	13.3
Fabaceae	*Cassia fistula*	Golden shower tree	17.3

[a](Dry weight of methanol extract/dry weight of test leaves)×100

formula suggested by Vincent (1947). Each experiment (all concentrations for each treatment) was repeated three times. The inhibition percentage was calculated as shown in Eq. 1

$$\% \text{ CDI} = \text{A} - \text{B}/\text{A} \times 100 \quad (1)$$

Where A=in controls and B=the radial growth of treated fungal cultures; and B=the radial growth of the tested fungus in treatment.

Efficacy of plant extracts against *S. rolfsii* in sugar beet under greenhouse conditions

Seeds of sugar beet (Kawemira variety) were treated with plant extracts by two methods with the most effective concentration of each plant extract under laboratory conditions (200 ppm). In the first method, the crude extracts were diluted in water to the most effective concentration under laboratory conditions (200 ppm) for each extract, and separate seed lots were soaked in this concentration for 8 h. In the second method, seeds were moistened with the required concentration of aqueous plant extracts. Then, talc powder and few drops of gum were added to assist in coating the seeds, which were subsequently air-dried.

At the greenhouse of Sakha Research Station in Kafrelsheikh, Agriculture Research Centre, Cairo, Egypt, the seeds were sown in sterilized 35-cm-diameter pots filled with sandy clay soil previously sterilized with 5% formalin. Each pot was filled with 5 kg soil and the soil infested with the tested fungus at a rate of 2% fungus to soil (w/w). The soil was then moistened with water for 1 week before seed treatment and sowing. Sugar beet seeds were soaked or coated in the tested plant extracts at the most effective concentration under laboratory conditions (200 ppm), and then 15 seeds were sown in each pot. The synthetic fungicide (thiram+carboxin) was incorporated in a seed coating using the talcum method or as a seed treatment by soaking at a rate of 2 g/kg seeds as a reference compound for this disease control. Plant growth was recorded after 15 and 45 days. The percentage of plants affected by damping-off was also estimated according to the scale adopted by Grainger (1949). Seeds soaked only in distilled water served as controls for the soaking application. Sugar beet seeds that were moistened with water and a few drops of Arabic gum were used as a control for the coating treatment. Survival percentages (efficacy of each treatment relative to un-infected control) after 15 days (evaluation of pre-emergence stage) and 45 days (evaluation of post-emergence stage) of treatment were calculated as shown in Eq. 2.

$$\% \text{ Survival} = \text{NOUP}/\text{TPN} \times 100 \quad (2)$$

Where NOUPis thenumber of un-infected plants, and TPN is the total plant number

Chemical composition of the most effective plant extracts

GC-MS analysis was performed to identify the components of the most effective plant extracts (*T. populnea* var. *acutiloba* and *Chrysanthemum frutescens*) according to the method described by Duarte-Almeida et al. (2004). The analysis was conducted on an HP 6890 GC system coupled with a 5973 network mass selective detector with an HP-5MS capillary column (60 m×0.25 mm, film thickness 0.25 μm). The oven temperature program was initiated at 50°C, held for 2 min and subsequently raised to 200°C at a rate of 5°C min^{-1}. Helium was used as the carrier gas at a flow rate of 1.0 ml min^{-1}, with a split ratio equal to 1/50. The injector and detector temperatures were 250 and 200° C, respectively. Some of the detected compounds in the tested plant extracts were identified by comparison of their retention indices (RI) and mass spectra fragmentation with the available analytical standards: tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, octadecanoic acid, linalool, 1,8-cineole and 9,12,15-octadecanoic acid. They were also identified by comparison of their RI and mass spectra fragmentation with those stored in the Wiley and NIST libraries associated with GC-MS. Several other compounds could be identified only through the second method. The samples were analyzed by the Central Laboratory for Pesticides, Agriculture Research Centre, Cairo, Egypt.

Toxicity assessment

Toxicity assessments were performed using 8-week-old 80–100 g Wistar male rats *(Rattus norvegicus*) obtained from the Faculty of Medicine, Tanta University, Egypt. Wister rats were housed in wire cages under standard conditions with free access to drinking water and food. The rats were kept in a temperature-controlled room with 14 h light and 10 h dark cycles. The rats were given a standard diet as described by Romestaing et al. (2007). Before treatment, the rats were maintained normally for 2 weeks during feeding for adaptation. The rats were divided randomly into three groups, each comprising three animals. Two groups were subjected to the treatment with the most effective plant extracts and the third group served as a control. The most effective plant extracts (*T. populnea* var. *acutiloba* and *Chrysanthemum frutescens*) were administered to rats orally at a concentration of 500 mg/kg body weight. Control group rats were orally administrated an equal amount of almond oil. After 21 days of treatment, the rats were sacrificed under anesthesia. Specimens from lung and liver were taken from each treatment and kept in 10% neutral buffered formalin for histopathological tests. The histopathology tests were carried out at the Histopathology Laboratory, Department of Histopathology, Faculty of Veterinary Medicine, Kafr El-Sheikh University according to the method described by Bancroft and Stevens (1996).

Statistical analysis

Data were subjected to the analysis of variance test and Newman-Keuls's multiple range test using a computer program SAS (Version 6.12, SAS Institute, Cary, NC).

Results

Efficacy of the tested plant extracts against *S. rolfsii* under laboratory conditions

The leaf extracts inhibited the radial growth of *S. rolfsii* significantly compared to the control. The leaf extract of *T. populnea* var. *acutiloba* was the most effective against *S. rolfsii*, with an inhibition percentage of 82.8%, followed by *Chrysanthemum frutescens*, *Caesalpinia gilliesii*, *E. japonicus*, *Cassia senna*, *B. purpurea* and *Cassia fistula* with inhibition percentages of 79.5, 78.3, 78.0, 77.2, 75.0 and 74.0%, respectively (Table 2). However, the standard fungicidal treatment against *S. rolfsii* (thiram+carboxin) was still the most effective treatment compared to all plant extracts. The efficacy of the tested plant extracts was dose-dependent, since the toxicity against *S. rolfsii* increased as their concentration increased.

Table 2 Efficacy of plant extracts against damping-off of sugar beet caused by *Sclerotium rolfsii* under laboratory conditions

Treatment	Extract concentration (ppm)	Inhibition percentage (%)
Cassia senna	50	16.50 m*
	100	34.50 k
	150	57.80 gh
	200	78.30 e
Caesalpinia gilliesii	50	22.50 kl
	100	41.30 i
	150	61.00 g
	200	79.50 de
Thespesia populnea var. *acutiloba*	50	21.00 l
	100	52.80 h
	150	67.00 fg
	200	82.80 d
Chrysanthemum frutescens	50	20.70 l
	100	53.25 h
	150	63.40 g
	200	79.00 de
Euonymus japonicus	50	21.00 l
	100	34.20 k
	150	65.20 g
	200	78.00 e
Bauhinia purpurea *B. purpurea*	50	5.00 o
	100	15.00 m
	150	53.30 h
	200	75.00 f
Cassia fistula	50	9.80 n
	100	34.50 k
	150	38.80 j
	200	74.00 f
Thiram+Carboxin	1	98.00 a
	10	90.70 c
	25	94.50 b
	50	94.50 b
Control	0.00	0.00 p

*Lower case letters in this column indicate separation of means according to the Student Newman Keuls multiple range test ($P<0.05$)

Efficacy of the tested plant extracts against *S. rolfsii* under greenhouse conditions

Table 3 and Figs. 1 and 2 show the relative efficacy of the plant extracts and the synthetic fungicide against *S. rolfsii* under greenhouse conditions. Among seed-soaking treatments, thiram+carboxin was the most effective treatment against *S. rolfsii* , followed by *T. populnea* var. *acutiloba*, *Chrysanthemum frutescens*, *Caesalpinia gilliesii*, *Cassia senna*, *E. japonicus*, *Cassia fistula* and *B. purpurea* extracts. The survival percentages of sugar beet plants after

Table 3 Efficacy of plant extracts (200 ppm) against *S. rolfsii* by either soaking or coating sugar beet seeds relative to thiram+carboxin (2 g/kg) under greenhouse conditions

Treatment	No. seedling survival			
	Coating treatment		Soaking treatment	
	Pre-emergence 15 days	Post emergence 45 days	Pre-emergence 15 days	Post emergence 45 days
Cassia senna	25.0 f*	21.9 e	20.5 f	17.7 ef
Caesalpinia gilliesii	27.9 e	24.5 d	25.5 d	21.6 d
Thespesia populnea var. *acutiloba*	30.1 d	28.6 c	28.45c	25.4 c
Chrysanthemum frutescens	33.1 c	28.8 c	28.8 c	24.4 c
Euonymus japonicus	25.8 f	21.6 e	23.4 e	21.1 d
Bauhinia purpurea	21.5 g	19.1 g	20.4 f	19.1 e
Cassia fistula	22.7 g	20.0 f	20.4 f	16.0 f
Thiram+carboxin	35.9 b	31.1 b	31.8 b	28.1 b
Control (infected)	9.9 h	9.0 h	7.6 g	5.1 g
Control (non-infected)	44.6 a	40.5 a	38.5 a	31.4 a

*Lower case letters in this column indicate separation of means according to the Student Newman Keuls multiple range test ($P<0.05$)

treatment were 68.2, 64.1, 63.9, 58.9, 48.4, 48.2, 44.5 and 42.9% for the above mentioned treatments, respectively (Fig. 1).

Thiram+carboxin was the most effective seed-coating treatment against *S. rolfsii*, followed by *T. populnea* var. *acutiloba*, *Chrysanthemum frutescens*, *Caesalpinia gilliesii*, *E. japonicus*, *Cassia senna*, *B. purpurea* and *Cassia fistula* extracts, respectively. The survival of sugar beet plants after treatment was 71.0, 64.2, 60.9, 5, 53.4, 48.8, 44.3 and 38.9 %, respectively, for the above-mentioned treatments (Fig. 2).

Moreover, the respective efficacy of plant extracts versus the synthetic pesticide was, in both cases, lower than efficacy after 45 days for both methods of seed treatment (Table 3; Figs. 1, 2). Generally, the efficacy of *Chrysanthemum frutescens*, *Caesalpinia gilliesii*, *Cassia senna* and *Cassia fistula* extracts against *S. rolfsii* was higher when the sugar beet seeds were soaked rather than coated. However, the remaining four plant extracts and the synthetic fungicide each had lower efficacy when sugar beet seeds were soaked rather than coated. There was no observed phytotoxicity of the plant extracts on the sugar beet seedlings.

Composition of the most effective plant extracts

The compounds identified in the most effective botanical extracts (*T. populnea* var. *acutiloba* and *Chrysanthemum frutescens*) against *S. rolfsii* are illustrated in Tables 4 and 5. A total of 25 compounds were identified from *T. populnea*

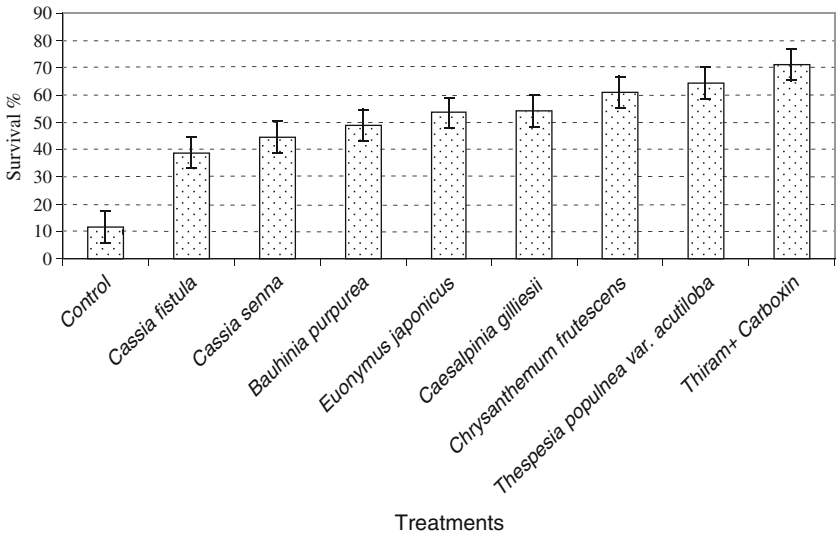

Fig. 1 Survival percentages [mean±standard error (SE)] of sugar beet plants infested with *Sclerotium rolfsii* subsequent to various seed-soaking treatments after 45 days under greenhouse conditions

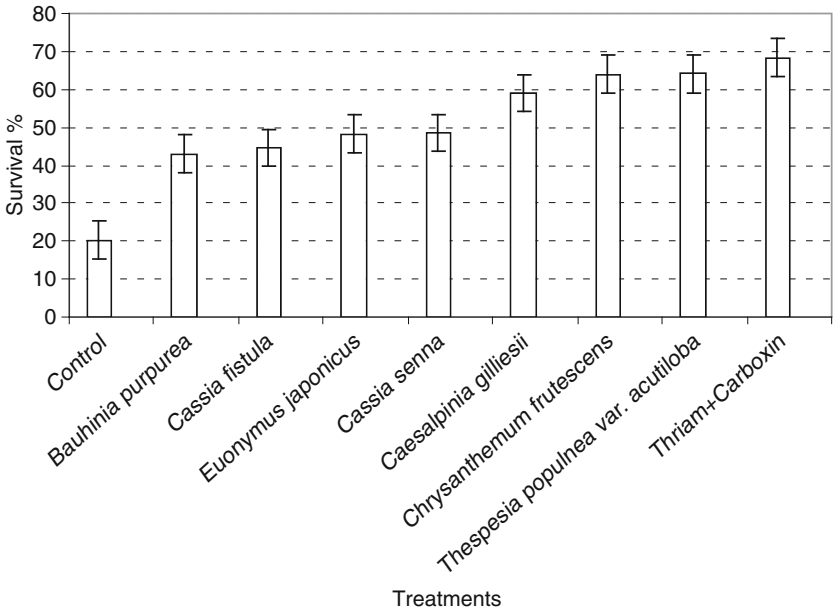

Fig. 2 Survival percentages (mean± SE) of sugar beet plants surviving infestation with *S. rolfsii* subsequent to various seed-coating treatments after 45 days under greenhouse conditions

var. acutiloba extract, while 12 compounds were identified from *Chrysanthemum frutescens* extract (Tables 4, 5). The identified compounds included aldehydes, esters, alcohols and fatty acids.

Toxicity evaluation

The normal structure of rat lung tissue is shown in Fig. 3a. For rats treated with *T. populnea* var. *acutiloba* extract at

Table 4 The main constituents of *Thespesia populnea* var. *acutiloba* extract determined by gas chromatography-mass spectroscopy (GC-MS) analysis

No.	Compound	Retention time (min)	Area (%)
1	Cyclohexanone (dimethyl acetal)	5.02	16.81
2	Methoxy propoxy 2-propanol	5.19	8.51
3	Cyclohexasiloxane	7.96	0.94
4	1,6,10-Dodecatriene,7,11-methyl-3-methylene	9.24	0.25
5	Cycloheptasiloxane (tetradecamethyl)	9.47	2.2
6	Diethyl phthalate	10.51	0.53
7	Benzene,(1-butylheptyl)	10.72	0.57
8	Benzene,(1-methyldecyl)	11.32	0.18
9	Tetradecanoic methyl ester	11.43	0.38
10	2,6,10-Dodecatrien-1-ol,3,7,11-trimethyl	11.52	0.79
11	Benzene,(1-butyloctyl)	11.57	0.36
12	Tetradecanoic acid	11.91	1.09
13	Cyclononasiloxane	12.26	1.78
14	Isopropyl myristate	12.36	0.61
15	Neophytodiene	12.51	2.51
16	Loliolide	12.59	0.93
17	Pentadecanoic acid	13.41	1.91
18	N-hexadecanoic	14.19	14.54
19	9,12,15-Octadecanoic-methyl-ester	15.56	3.15
20	Phytol	15.74	5.02
21	9,12,15-Octadecatrien-1-ol	16.38	11.97
22	9,12,15-Octadecanoic acid	16.42	3.72
23	Octadecanoic acid	16.6	3.09
24	Di-n-octyl phthalate	21.69	3.77
25	Vitamin E	29.84	0.51

Table 5 Main constituents of *Chrysanthemum frutescens* extract determined by GC-MS analysis

No.	Compound	Retention time (min)	Area (%)
1	1,8-Cineole	4.98	13.33
2	Linalool	5.70	8.18
3	Cyclohexasiloxane (dodecamethyl)	4.74	1.34
4	Terpinyl acetate	8.14	21.6
5	2,6-Octadien-1-ol3,7dimethyl	8.38	1.44
6	1,6,10-Dodecatriene,7,11-methyl-3-methylene	9.02	3.83
7	Cycloheptasiloxane (tetradecamethyl)	3.25	3.68
8	Cyclononasiloxane (octadecamethyl)	11.97	2.86
9	Cyclohexadecane	12.65	2.48
10	1,9-Tetradecadiene	14.6	4.34
11	Iron monocarbonyl −1,3 butadiene 1,4 dicarbonic acid diethyl ester a,a dipyridyl	26.75	3.27
12	Tetradecanoic acid octadecyl ester	27.08	6.13

dose of 500 mg/kg, the tissue was somewhat similar to control samples and displayed a small amount of infiltration by lymphocytes and an increase in cell mass (Fig. 3b). For rats treated with *Chrysanthemum frutescens* extract at the same dose level, the lung was observed to be as normal as the control with some diffusion of blood and mild thickening (Fig. 3c).

The normal structure of liver tissue is shown in Fig. 4a. In rats treated with *T. populnea* var. *acutiloba* at dose of 500 mg/kg, blood vessels appeared to be engorged with blood and hepatocytes contained vacuolated cytoplasm (Fig. 4b). However, treated livers looked similar to controls. In the case of rats treated with *Chrysanthemum frutescens* at the same dose level, livers were obsesrved to appear as normal as controls. There were blood vessels engorged with blood and activation of Kopffer cells. A few instances of lymphatic infiltration and blood vessels engorged with blood were observed (Fig. 4c).

Discussion

Many plant extracts have been reported to have efficacy against *S. rolfsii* under either laboratory or greenhouse conditions (El-Shoraky 1998; Yossry et al. 1998; El-shahawy 2002; Eltoony 2003; Okemo et al. 2003). However, for the plant extracts assayed in this study, this is the first report of their efficacy against *S. rolfsii*.

It was observed that, among the compounds identified from extracts of *Chrysanthemum frutescens* and *T. populnea* var.

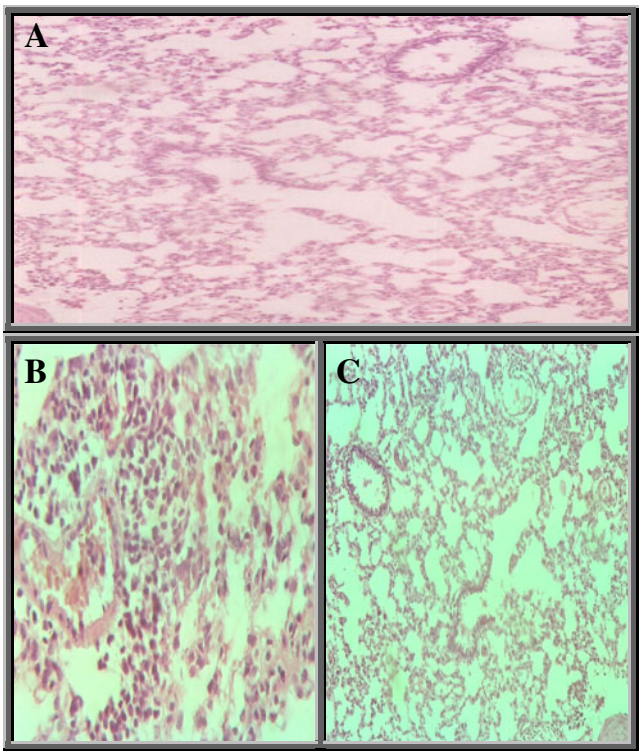

Fig. 3a–c Sections from rat lungs, 21 days after treatment with plant extracts at a dose level of 500 mg/kg. **a** Control, **b***Thespesia populnea* var. *acutiloba*, **c** *Chrysanthemum frutescens*

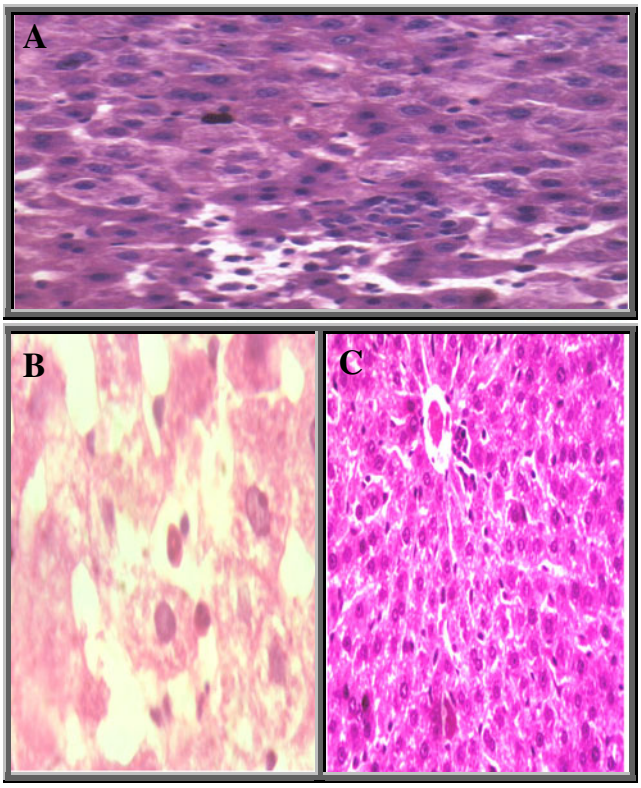

Fig. 4a–c Sections from rat livers, 21 days after treatment with plant extracts at a dose level of 500 mg/kg. **a** Control, **b***Thespesia populnea* var. *acutiloba*, **c** *Chrysanthemum frutescens*

acutiloba, constituents such as tetradecanoic acid; tetradecanoic acid; pentadecanoic acid; N-hexadecanoic acid; hexadecanoic acid; phytol; linalool; 1,8 cineole and 9, 12, 15 octadecanoic acid were detected with high percentages relative to other detected compounds. The antifungal activity of *Chrysanthemum frutescens* and *T. populnea* var. *acutiloba* extracts against *S. rolfsii* may be due to the presence of these fatty acids and their derivatives (Hammer et al. 2003; Walters et al. 2004; Wagh et al. 2007; Tzakou et al. 2001; Cheraif et al. 2007; Chutia et al. 2009; Kelen and Tepe 2008; Soković et al. 2009; Ahmadi et al. 2010). Moreover, the efficacy of the most effective plant extracts at higher concentrations might actually be comparable to chemical pesticides. In fact, the actual dosage of any one compound identified in these extracts could be relatively low, safe, and economically feasible.

Although the antimicrobial activity of plant extracts is attributed mainly to their major components, the synergistic or antagonistic effect of minor components such as loliolide (0.93%) has to be considered because they have known antifungal activity (Ragas et al. 2002). Therefore, each component of the plant extract may potentially make a unique contribution to their activity.

Under greenhouse conditions, it was observed that the efficacy of *Chrysanthemum frutescens* extract against *S. rolfsii* was slightly higher than extracts of *T. populnea* var. *acutiloba*. This may be due to the presence of known bioactive compounds such as 1,8 cineole (33.33%), linalool (8.18%) and terpinyl acetate (21.6%), with higher percentages occurring in the extract of *Chrysanthemum frutescens* versus *T. populnea* var. *acutiloba* (Tables 4, 5).

Botanical extracts as pest control agents present two main characteristics: the first is their safety to humans and the environment, and the second is a lower likelihood of resistance developing within the pathogen of concern. Regarding safety, the toxicity evaluation of most effective plant extracts revealed that there were some slight variations that occurred sporadically in treated rats relative to control with respect to the histopathology of treated organs. Moreover, the observed changes in tissues were mostly uncorrelated with dosage, which potentially indicates the safety of these plant extracts in the context of human health. Also, the rat tests are often more sensitive and may not reflect human sensitivity. Moreover, the exposure levels may be far greater than what would actually be experienced or detected in sugar beet crops after they are grown and processed.

The use of essential oils in antimicrobial agents is considered to present relatively low risk where the development of resistance in pathogenic organisms is concerned. Concerning resistance development, it is believed that it is difficult for the pathogen to develop resistance to such a mixture of bioactive components with apparently different mechanisms of antimicrobial activity (Liu et al. 2008).

This study implies the effectiveness of the tested plant extracts as an alternative to synthetic fungicides for controlling a major damping-off pathogen of sugar beet. The use of control measures based on these extracts has the potential to reduce environmental pollution and the adverse effects on human health that are a risk where synthetic pesticides are used.

Conclusions

The tested plant extracts can be considered a natural source of fungicidal material potentially useful for the control of *S. rolfsii* in sugar-beet crop. Antifungal activity was confirmed in all of the assayed plant species, despite some variation in their efficacy against damping-off. In vivo results under greenhouse conditions confirmed that these plant extracts can be used as a viable and safe alternative for controlling *S. rolfsii*. Further research on the practical effectiveness of non-phytotoxic plant extracts or essential oils for plant protection is needed.

References

Ahmadi F, Sadeghi S, Modarresi M, Abiri R, Mikaeli A (2010) Chemical composition, in vitro anti-microbial, antifungal and antioxidant activities of the essential oil and methanolic extract of *Hymenocrater longiflorus* Benth of Iran. Food Chem Toxicol 48:1137–1144

Aycock R (1966) Stem rot and other diseases caused by *Sclerotium rolfsii*. N C Agric Exp Stn Tech Bull 174:202

Bancroft JD, Stevens A (1996) Theory and practice of histopathological techniques, 4th edn. Churchill Livingstone, New York, NY

Cheraif I, Ben Jannet H, Hammami M, Khouja ML, Mighri Z (2007) Chemical composition and antimicrobial activity of essential oils of *Cupressus arizonica* Greene. Biochem Syst Ecol 35:813–820

Chutia M, Bhuyan PD, Pathak MG, Sarma TCP, Boruah P (2009) Antifungal activity and chemical composition of *Citrus reticulata* Blanco essential oil against phytopathogens from North East India. LWT Food Sci Technol 42:777–780

Cramer RA, Byrne PF, Brick MA, Panella L, Wickliffe E, Shchwartz HF (2003) Characterization of *Fusarium oxysporum* isolates from common bean and sugar beet using pathogenecity assays and random amplified polymeric DNA markers. J Phytopathol 151:352–360

Cornelius CE, Bishop J, Switzer J, Rhode EA (1959) Serum and tissue transaminase activities in domestic animals. Cornell Vet 49:116–121

Duarte-Almeida JM, Negri G, Salatino A (2004) Volatile oils in Leaves of *Bauhina* (Fabaceae Coesaplinioideae). Biochem Syst Ecol 32:747–753

El-Abyad MS, Abu-taleb AM, Abdel-Mawgoud T (1997) Response of host cultivar to cell wall-degrading enzymes of the sugarbeet pathogens *Rhizoctonia solani* Kühn and *Sclerotium rolfsii* Sacc. under salinity stress. Microbiol Res 152:9–17

Elad Y (1995) Mycoparasitism. In: Kohmoto K, Singh US, Singh RP (eds) Pathogenesis and host specificity in plant diseases:

histopathological, biochemical, genetic and molecular bases, vol II, eukaryotes. Pergamon, Oxford, pp 285–307

El-Shoraky FSA (1998) Using extracts and oils of some plant diseases. Tanta University, Egypt, Dissertation

Eltoony AME, Awad NGH, Tadrous MFE, Ahmed FS (2003) Chemical and biological control of tomato damping-off disease under nursery conditions with special references to the antagonism between the causal pathogens. Egyptian J Appl Sci 18:47–68

El-shahawy EA (2002) Biocidal effect of some compounds on some soil borne fungi. Tanta University, Egypt, Dissertation

Eweis M, Elkholy SS, Elsabee MZ (2006) Antifungal efficacy of chitosan and its thiourea derivatives upon the growth of some sugar-beet pathogens. Int J Biol Macromol 38:1–8

Gouda MI (2001) Studies on some casuals of sugar beets root rot. Tanta Universty, Egypt, Dissertation

Grainger J (1949) Crop and diseases. Plant pathology Department, West of Scotland Agriculture, College Research Bull 9:51

Hammer KA, Carson CF, Riley TV (2003) Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. J Appl Microbiol 95:853–860

Kelen M, Tepe B (2008) Chemical composition, antioxidant and antimicrobial properties of the essential oils of three *Salvia* species from Turkish flora. Bioresour Technol 99:4096–4104

Latha P, Anand T, Ragupathi N, Prakasam V, Samiyappan R (2009) Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. Biol Control 50:85–93

Liu W-W, Mu W, Zhu B-Y, Du Y-C, Liu F (2008) Antagonistic activities of volatiles from four strains of *Bacillus* spp. and *Paenibacillus* spp. against soil-borne plant pathogens. Agric Sci China 7:1104–1114

Mdee LK, Masoko P, Eloff JN (2009) The activity of extracts of seven common invasive plant species on fungal phytopathogens. South Afr J Bot 75:375–379

Mengs U, Mitchell J, McPherson S, Gregson R, Tigner J (2004) A 13-week oral toxicity study of senna in the rat with an 8-week recovery period. Arch Toxicol 78:269–275

Mitchell JM, Mengs U, McPherson S, Zijlstra J, Dettmar P, Gregson R, Tigner JC (2006) An oral carcinogenicity and toxicity study of senna (*Tinnevelly senna* fruits) in the rat. Arch Toxicol 80:34–44

Okemo OP, Baisa HP, Jorge M, Vivancoa JM (2003) *In vitro* activities of *Maesa lanceolata* extracts against fungal plant pathogens. Fitoterapia 74:312–316

Panda S, Kar A (1999) *Withania somnifera* and *Bauhinia purpurea* in the regulation of circulating thyroid hormone concentrations in female mice. J Ethnopharmacol 67:233–239

Park SH, Ko SK, Chung SH (2005) *Euonymus alatus* prevents the hyperglycemia and hyperlipidemia induced by high-fat diet in ICR mice. J Ethnopharmacol 102:326–335

Punja ZK (1985) The biology, ecology, and control of *Sclerotium rolfsii*. Annu Rev Phytopathol 23:97–127

Ragas CY, Hofilena JG, Rideout JA (2002) New furanoid diterpenes from *Caesalpinia pulcherrima*. J Nat Prod 65:1107–1110

Romestaing C, Piquet M, Bedu E, Rouleau V, Dautresme M, Hourmand-Ollivier I, Filippi C, Duchamp C, Sibille B (2007) Long term highly saturated fat diet does not induce NASH in Wistar rats. Nutr Metab 4:4. doi:10.1186/1743-7075-4-4

Soković MD, Vukojević J, Marin PD, Brkić DD, Vajs V, Griensven LLD (2009) Chemical composition of essential oils of *Thymus* and *Mentha* species and their antifungal activities. Molecules 14:238–249

Tzakou O, Pitarokili D, Chinou IB, Harvala C (2001) Composition and antimicrobial activity of the essential oil of *Salvia ringens*. Planta Med 67:81–83

Vincent JH (1947) Distortion of fungal hyphae in presence of certain inhibitors. Nature 159:850–850

Yossry AA, Abedalal SM, El-Imery SM (1998) Fungi toxic properties of some plant extracts against the growth of soil borne disease fungi. Ann Agric Sci Moshtohor 3:891–909

Wagh P, Rai M, Deshmukh SK, Durate MCT (2007) Bioactivity of oils of *Trigonella foenum-graecum* and *Pongamia pinna*. Afr J Biotechnol 6:1592–1596

Walters D, Raynor L, Mitchell A, Walker R, Wallker K (2004) Antifungal activity of four fatty acids against plant pathogenic fungi. Mycopathology 157:87–90