

RESEARCH PAPERS

Alternative control of early blight of tomato using plant extracts from *Acacia nilotica*, *Achillea fragrantissima* and *Calotropis procera*

ZAKARIA A.M. BAKA¹ and YOUNES M. RASHAD²¹ Department of Botany, Faculty of Science, University of Damietta, New Damietta, Egypt² Plant Protection and Biomolecular Diagnosis Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt

Summary. The *in vitro* and *in vivo* antifungal potential of extracts of three wild medicinal plants, (*Acacia nilotica* (L.) Delile, *Achillea fragrantissima* (Forssk.) Sch.Bip. and *Calotropis procera* (Aiton) W. T. Aiton) was examined against *Alternaria solani*, the causal agent of the early blight of tomato. Aqueous or ethanol extracts of all tested plants reduced the mycelial growth and conidium germination of *A. solani* *in vitro*. Ethanol extracts were more effective against the pathogen than the aqueous extracts. Extract of *C. procera* exhibited more antifungal potential against the pathogen than other plant extracts. Observations by scanning and transmission electron microscopy showed dramatic alterations in the morphology and ultrastructure of *A. solani* when treated with the ethanol extract of *C. procera* at a concentration of 20%. Phytochemical screening confirmed the presence of many bioactive constituents in the extracts which were in greater amounts in *C. procera* than the other two plants. In a plot experiment, both types of extracts from *C. procera* reduced disease severity. Tomato fruit yield was increased after the treatment with the plant extracts.

Key words: *Alternaria solani*, antifungal activity, electron microscopy.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetables in the world, including Egypt. Early blight, caused by *Alternaria solani* (Ell. and Mart.) Jones and Grout., is one of the common foliar diseases which results in yield losses up to 70% (Glala *et al.*, 2005). Control of this disease is heavily reliant on multiple applications of chemical fungicides during flowering and fruiting (Akila *et al.*, 2012). Furthermore, the use of synthetic pesticides to control fungal plant diseases of food commodities is restricted by their possible carcinogenicity, high and acute toxicity, long degradation periods, and potential environmental pollution.

The use of compounds in plant extracts may be an alternative to currently use chemical fungicides

for controlling phytopathogenic fungi, because they constitute a rich source of bioactive chemicals such as phenols, flavonoids, quinones, tannins, alkaloids, saponins and sterols (Isman, 2000). Since these extracts can be active against fungal phytopathogens, and are biodegradable to nontoxic products, they are potentially suitable for use in integrated pest management programmes (AL-Askar and Rashad, 2010; AL-Askar *et al.*, 2014; Khafari *et al.*, 2014).

Egypt has about 384 different species of medicinal plants found in the Mediterranean coastal region, deserts, oases and Sinai Peninsula. The Egyptian flora is rich in its diversity, with hundreds of plants used in traditional medicine since antiquity, when the ancient Egyptians recorded thousands of plants and their uses in the Ebers papyrus (Saleh, 2003; Shafik and Elseesy, 2003).

Acacia nilotica (L.) Delile, (Egyptian thorn; gum Arabic tree), is a multipurpose tree legume. It is widespread in subtropical and tropical Africa and in

Corresponding author: Y.M. Rashad
E-mail: younesrashad@yahoo.com

Asia. The plant is traditionally and widely used for the treatment of various ailments. Satish *et al.* (2007) recorded antifungal activities of methanol and aqueous extracts of *A. nilotica* against important seed-borne *Aspergillus* sp. *Achillea fragrantissima* (Forssk.) Sch.Bip. (Lavender cotton; Qaysūm) is a medicinal plant that has been used in folk medicine and for pharmaceutical purposes since ancient times. Also-haili and Al-fawwaz (2014) recorded varied antifungal activities of *A. fragrantissima* essential oil against three food spoilage fungi (*i.e.* *A. nigar*, *Penicillium* sp. and *Rhizopus* sp.). *Calotropis procera* (Aiton) W. T. Aiton (milk weed; osher), is a glabrous laticiferous shrub or small tree, which has been used in Egypt as a folk medicine (Shafik and Elseesy, 2003). Leaves, flowers and methanol extracts from latex of *C. procera* showed fungicidal activities against *Candida albicans*, *C. tropicalis*, *P. chrysogenum* and *Saccharomyces cerevisiae* (Nenaah and Ahmed, 2011).

The search for plant extracts from medicinal plants to control plant pathogens has increased during the last few years, and efficacy of plant extracts against *A. solani* has been demonstrated in many countries (Suleiman, 2010; Sallam, 2011; Sallam and Abo-Elyousr, 2012; Maya and Thippanna, 2013; Ravikumar and Garampalli, 2013; Khafari *et al.*, 2014).

The aim of present study was to evaluate aqueous and ethanol extracts from three Egyptian wild medicinal plants (*A. nilotica*, *A. fragrantissima* and *C. procera*) collected from the Sinai Peninsula, for the management of *A. solani*, the causal agent of the early blight of tomato.

Materials and methods

The pathogen

Alternaria solani was isolated from naturally infected tomato leaves showing the early blight symptoms. The pathogen was isolated using potato dextrose agar (PDA) and V8 media, and identified in the Botany Department, Faculty of Science, Damietta University, Egypt.

Plant materials and extract preparation

Three wild medicinal plants (*A. nilotica*, *A. fragrantissima* and *C. procera*) were collected from different locations in the Sinai Peninsula, Egypt. Leaves were extracted using water or ethanol. The methods of extraction were applied according to Rivillas-Ace-

vedo and Soriano-García (2007). For aqueous extraction; 100 g of each air-dried sample of plant material was added to 1 L of distilled water, and extracted under cold conditions for 24 h. Plant extracts were filtered and this concentration (10%) was used as a crude aqueous extract. For ethanol extraction, a prepared fine powder (40 g) of dried plant material was macerated with 200 mL of ethanol (80%) for 24 h in a 500 mL capacity flask, and treated in an ultrasonic cleaner for 10 min. The mixtures were then centrifuged at 4000 g for 20 min. The supernatant was evaporated under reduced pressure at a temperature below 45°C. The residues were dissolved in 40 mL ethanol (50%) as original extracts and stored at 5°C in darkness.

Effects of plant extracts on linear mycelial growth

Crude leaf extracts of the three medicinal plants were tested *in vitro* for efficacy against *A. solani* mycelial growth using agar plate assay (Schmitz, 1930). The plant extracts were added to conical flasks containing sterilized PDA before solidification to obtain the final concentrations of 2.5, 5, 10 and 20% (v/v). After solidification, the plates were each inoculated with a 6 mm agar piece containing *A. solani* mycelia. For each crude leaf extract, the experiments were performed in three replicates. Plates containing mancozeb (1 mL at of concentration of 0.2%) and sterile distilled water were used, respectively, as positive and negative controls. The inoculated plates were incubated at 25°C, and the diameters of fungal colonies were measured each day for 5 d. The antifungal activity of the extracts was measured as the linear mycelial growth of the colonies of the pathogenic fungus.

Effects of plant extracts on conidium germination

Antifungal activity of aqueous and ethanol extracts on conidium germination of *A. solani* was tested using a microscope slide technique (Nair and Llingboe, 1962). The plant extracts (at concentrations of 2.5, 5, 10 and 20%) were added as films to dried clean slides. Then, conidia suspensions (0.1 mL containing 5×10^6 conidia) was spreaded over these films. Control treatments were prepared as films with sterilized distilled water. Three slides were used as replicates for each concentration. Each slide was placed on a glass rod in a Petri dish under moist conditions and incubated for 24 h at 25°C. Four microscopic slides (at 400x magnifica-

tion) were used for each slide. Mancozeb (commercial fungicide at 0.2%) and sterile distilled water were used, respectively, as positive and negative controls. The percentage of germinated conidia was calculated.

Electron microscopy

Because the ethanol extract of *C. procera* was the most effective treatment for reduction of mycelial growth of *A. solani*, a culture of the pathogen, of the same age as used in the control and extract treatments (20%) was prepared for Scanning (SEM) and transmission (TEM) electron microscopy. For the SEM preparation, mycelial plugs (1 × 1 × 3 mm) were fixed in phosphate-buffered 3% glutaraldehyde at pH 6.8, and then dehydrated in a crescent acetone series (30, 50, 70, 85, 95 and 100%). The samples were then critical point dried (Polaron CPD 7501, UK). The specimens were sputter-coated with platinum using a JFC-1600 autofine coater (Polaron SC7620). Fungal hyphae were examined using a Joel SEM-6400JSM-6360LV microscope (JEOL Ltd., Japan). For TEM preparation, samples were fixed in phosphate-buffered 3% glutaraldehyde at pH 6.8, post-fixed in phosphate-buffered 1% Osmium tetroxide and then dehydrated in a crescent ethanol series following the procedure described by Alberto *et al.* (1997). Mycelial plugs were embedded in plastic epoxy resin. Ultra-thin sections were cut with a Reichert ultramicrotome, stained with uranyl acetate followed by lead citrate, and examined with JEOL model JEM-1230 TEM.

Determination of Minimum Inhibitory Concentration (MIC)

Different concentrations (0.01, 0.02, 0.03, 0.06 and 0.08 µg mL⁻¹) of the extracts were prepared. Each of these was added to 18 mL of potato dextrose broth in test tubes. Each tube was then inoculated with 0.1 mL of conidium suspension of *A. solani* diluted to give a final conidium suspension of 10⁶ mL⁻¹. The tubes were incubated at 28 ± 2°C and examined for growth after 7 d. The least concentration of the plant extracts that permitted visible growth of the inoculated test organism in the broth medium was regarded as the MIC in each case. Control experiments were performed without the plant extracts.

Qualitative analysis of phytochemical constituents

Qualitative studies for tannins, saponins, flavonoids, cardiac glycosides, alkaloids and phenols were carried out according to Mir *et al.* (2013). Based on the intensity of colouration or the precipitates formed during the test, bioactive constituents were characterized as strongly present (+++), present (++), weakly present (+), and absent (-) when the test result was negative.

Field experiments

Egyptian tomato seeds (type Heirloom), used in this study were obtained from the Ministry of Agriculture, Egypt. The field trials were conducted twice over two growing seasons (2013 and 2014) at the Experimental Farm of the Faculty of Science, Damietta University, Damietta, Egypt. Field plots (3 × 3.5 m) each comprised two rows and five plants per row, and were arranged in a completely randomised block design. Three plots were used as replications for each treatment. Fifty-d-old plants were separately sprayed four times at 15 d intervals with the aqueous or ethanol plant extracts (30 mL at 20% concentration). Three other plots were sprayed with the chemical fungicide (Mancozeb, 30 mL, 0.2% concentration) as positive controls. Another three plots were sprayed with water to serve as negative controls. Two days after the second spraying, tomato seedlings were inoculated with a conidium suspension of *A. solani* (20 mL at concentration of 5 × 10⁶ conidia mL⁻¹). All plants were sprayed until run-off.

Disease severity

Disease severity was estimated 7 d after the final spray. Ten plants were taken from each treatment, and leaves of each plant were used to determine the disease severity. Infected leaves were classified into five categories (0, 1, 2, 3 and 4) according to the blighted area of leaves (Cohen *et al.*, 1991), i.e. 0 = no infected leaves, 1 = 25% or less, 2 = 26–50%, 3 = 51–75%, or 4 = 76–100% leaf area affected. Disease severity percentage was calculated according to the following formula:

$$\text{Disease severity (\%)} = \frac{\sum (n \times r_1) - (n \times r_4)}{4N} \times 100$$

Where n = Number of infected leaves, r₁ – r₄ = disease severity category and N = Total number of examined leaves.

Determination of fruit yield

After harvest, total weight of fruit per plant (g) following the different treatments of aqueous and ethanol plant extract applications were determined.

Statistical analyses

Data were analyzed with the statistical analysis software CoStat (version 6.4), for one way completely randomized block design. Duncan's multiple range test was used to compare means at a probability (P) level of ≤ 0.01 .

Results

Effect of plant extracts on mycelial growth of *Alternaria solani*

The effects of aqueous and ethanol extracts of *C. procera*, *A. nilotica* and *A. fragrantissima* *in vitro* tests on linear mycelial growth of *A. solani* is shown in Table 1. All of the treatments reduced linear mycelial growth of the pathogen compared with the experimental controls. The proportional reductions of mycelial growth increased with increasing concentrations of the extracts from 2.5 to 20%. Greater inhibition of growth was obtained with the ethanol extracts than the aqueous extracts. At concentration of 20%, ethanol extracts from *C. procera*, *A. nilotica* and *A. fragrantissima* gave, respectively, mean mycelial growth of 17.3, 18 and 21.3 mm. Aqueous extracts

from the plants gave, respectively, mean mycelial growth of 27.3, 28 and 31.7 mm.

Effects of plant extracts on conidium germination

All treatments significantly decreased the conidium germination (%) of *A. solani* compared with the control (Table 2). Ethanol extracts were more effective against reducing the conidium germination of the pathogen than aqueous extracts. The values of conidium germination were decreased with increasing the concentrations of extracts from 2.5 to 20%. The greatest reduction of germination (%) with aqueous or ethanol extracts was for those from *C. procera* followed by *A. nilotica* and *A. fragrantissima*. At concentration of 20% water extract, mean conidium germination was, respectively for extracts from the three plants, 13.5, 15.4, and 18.5%. For the ethanol extracts, germination was less, at means, respectively, of 11.1, 13.2 and 16.2 %.

Electron microscopy

SEM revealed that the hyphae of the pathogen exposed to plant extract were collapsed and curling, and short branching, whereas no such changes were noticed in the mycelia developed in the control treatment (Figure 1a,b). TEM observations revealed that the sub-cellular components of the pathogen in the control treatment were enclosed by a distinct electron-dense wall and a normal plasma lemma. Many

Table 1. Mean linear mycelial growth (mm) of *Alternaria solani* after 7 d incubation at 25°C, when exposed to aqueous (A) or ethanol (E) extracts from three medicinal plants at different concentrations .

Plant extract	Plant extracts concentration (%)							
	2.5		5		10		20	
	A	E	A	E	A	E	A	E
Control	66.3* a**	66.3 a	66.3 a	66.3 a	66.3 a	66.3 a	66.3 a	66.3 a
Mancozeb (0.2%)	15.6 e	15.6 d	15.6 d	15.6 d	15.6 d	15.6 d	15.6 d	15.6 d
<i>Calotropis procera</i>	36.3 d	24.0 c	33.2 c	22.2 c	29.0 c	19.0 c	27.3 c	17.3 cd
<i>Acacia nilotica</i>	39.2 c	25.1 c	34.7 bc	22.3 c	31.0 c	20.3 c	28.0 c	18.0 c
<i>Achillea fragrantissima</i>	41.7 b	28.5 b	37.3 b	26.6 b	33.7 b	24.3 b	31.7 b	21.3 b

* Each value represents the mean of three replicates.

** Values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P=0.01$).

Table 2. Mean percent germination of *Alternaria solani* conidia exposed to different concentrations of fungicide, or aqueous (A) or ethanol (E) extracts from three different plants at different concentrations.

Plant extract	Plant extracts concentration (%)							
	2.5		5		10		20	
	A	E	A	E	A	E	A	E
Control	80.3* a**	80.3 a	80.3 a	80.3 a	80.3 a	80.3 a	80.3 a	80.3 a
Mancozeb (0.2%)	10.8 e	10.8 e	10.8 d	10.8 d	10.8 d	10.8 d	10.8 d	10.8 d
<i>Calotropis procera</i>	33.5 d	30.7 d	27.4 c	26.6 c	26.0 c	22.4 c	13.5 c	11.1 d
<i>Acacia nilotica</i>	43.7 c	35.5 c	29.6 c	26.8 c	26.5 c	23.6 c	15.4 c	13.2 c
<i>Achillea fragrantissima</i>	54.5 b	49.4 b	48.5 b	44.2 b	38.5 b	29.1 b	18.5 b	16.2 b

* Each value represents the mean of three replicates.

** Values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P = 0.01$)

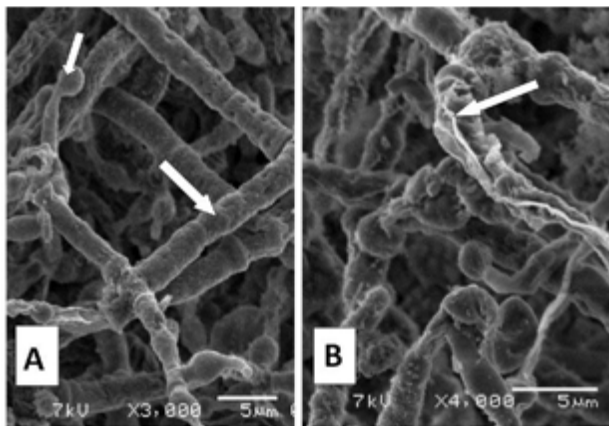


Figure 1. SEM micrographs showing effects of ethanol extract from *Calotropis procera* on the morphology of *Alternaria solani* hyphae. (a) Hyphae of control treatment (untreated) showing normal hypha (long arrow). Note immature conidium (short arrow). Bar = 10 μm . (b) Hyphae after the treatment with plant extract (20%). Note collapsed hypha (arrow). Bar = 5.0 μm .

organelles are observed in each cell, such as a nucleus surrounded by a double membrane and an endoplasmic reticulum (ER) associated with the nucleus, vacuoles, lipid droplets, Golgi bodies and mitochondria (Figure 2a). When the extract of *C. procera* was added to the growth medium of the pathogen, many ultrastructural changes of the mycelium occurred. These included electron-transparency of the cell walls, de-

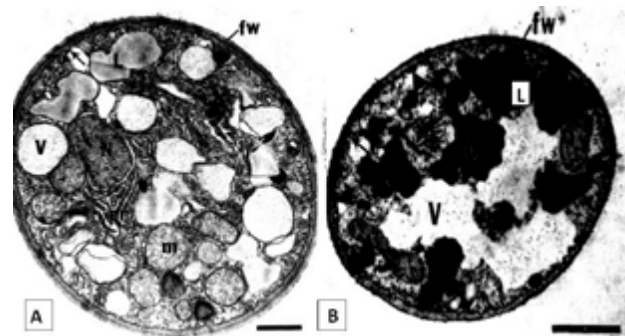


Figure 2. TEM micrographs showing the effect of ethanol extract of *Calotropis procera* on the ultrastructure of *Alternaria solani* hyphae. (a) A section of a hypha from untreated medium (control) showing an electron-opaque hyphal wall (fw) adjacent to the plasmalemma (arrow). Note the nucleus (N), numerous lipid bodies (L), Golgi bodies (g), endoplasmic reticulum (er), mitochondria (m) and numerous vacuoles (V). Bar = 0.5 μm . (b) A section of a hypha from medium amended with 20 % plant extract. Note the hyphal wall (fw), large electron-dense lipid bodies (L), large vacuoles (V). Note also the electron-dense material (arrowheads) deposited on the plasmalemma (arrow). Bar = 1.0 μm .

generation of cytoplasm and an increase in numbers of lipid droplets and vacuoles (Figure 2b).

Minimum Inhibitory Concentration (MIC)

MICs of ethanol plant extracts from the tested plants were less than those of aqueous extracts (Fig-

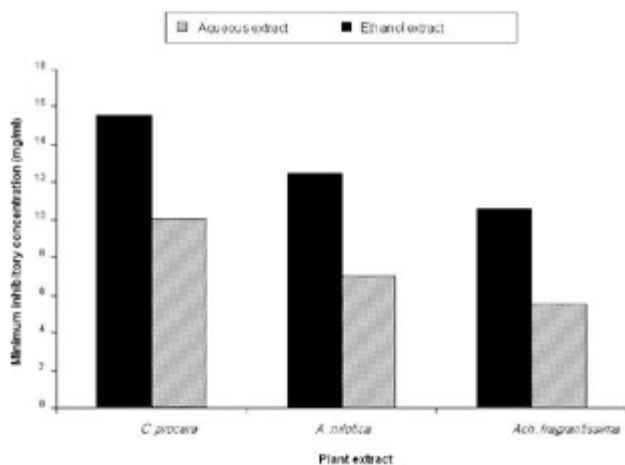


Figure 3. Minimum inhibitory concentrations (mg mL⁻¹) of the tested plant extracts.

ure 3). MICs for the ethanol extracts from the three different plants were, respectively, about 6, 8, and 10 mg mL⁻¹ for *C. procera*, *A. nilotica* and *A. fragrantissima*. MICs of aqueous extracts were, respectively, about 11, 12, and 16 mg mL⁻¹ (Figure 3).

Phytochemical constituents of plant extracts

Phytochemical screening for aqueous leaf extracts from the tested plants revealed the presence of flavonoids, saponins, tannins, alkaloids, cardiac glycosides and phenol. The ethanol leaf extracts contained also these phytochemicals, except that saponins were not detected in the extract from *C. procera* (Table 3). Flavonoids, alkaloids, and cardiac glycosides were present in high amounts in the extracts from *C. procera* as compared with the other plants.

Effects of plant extracts on disease severity in the field

Early blight severities (%) on tomato plants in the field, when treated with the tested plant extracts are shown in Table 4. All of the treatments reduced the disease severity compared with the control treatment. Mean early blight severity ranged from 13.2–23.7% for the aqueous plant extract treatments and 10.5–20.6% for the ethanol extracts, while in the control treatment gave mean severity of 53.2%. The aqueous and ethanol extracts of *C. procera* were the most effective against early blight, followed by the

Table 3. Qualitative analysis of phytochemical constituents of aqueous (A) or ethanol (E) extracts from leaves of three medicinal plants.

Phytochemical constituents		Plant extract		
		<i>Calotropis procera</i>	<i>Acacia nilotica</i>	<i>Achillea fragrantissima</i>
Flavonoids	A	+++*	++	+
	E	+	+	+
Saponins	A	+	+	+
	E	-	+	+
Tannins	A	+	+	+
	E	+	+	+
Alkaloids	A	+++	++	+
	E	++	+	+
Cardiac glycosides	A	+++	++	+
	E	+	+	+
Phenols	A	++	++	++
	E	+	+	+

* Strongly present (+++), present (++) , weakly present (+) and absent (-)

extracts from *A. nilotica* and *A. fragrantissima*. Aqueous extracts from the three plants, respectively, reduced the disease severity by 75, 70, and 56%, and equivalent reductions for the ethanol extracts were 80, 70, and 60%.

Effects of plant extracts on fruit yield

The effects of extracts from the three medicinal plants on fruit yield of tomato are shown in Table 5. Mean tomato fruit yields ranged from 374 to 481 g from the aqueous plant extract treatments, and 391 to 496 g from the ethanol extracts. compared with 258 g for the untreated plants. The aqueous and ethanol extracts plants increased fruit yield per plant comparing with the control. The greatest yields were obtained with extracts from *C. procera* followed by the extracts from *A. nilotica* and *A. fragrantissima*. Aqueous extracts from the three plants, respectively, increased fruit yield per plant about 86, 67 and 45%, while the ethanol extracts increased the yield per plant about 92, 77 and 54%. The yields followed a

Table 4. Mean early blight severity and proportional (%) disease reduction for tomato plants treated with fungicide, or aqueous or ethanol extracts from three medicinal plants, in a field trial.

Treatment	Aqueous extract		Ethanol extract	
	DS ^a (%)	DR (%)	DS (%)	DR (%)
Inoculated and not treated	53.2 ^b a ^c	0.0 d	53.2 a	0.0 c
Mancozeb (0.2%)	9.4 e	82.7 a	9.4 e	82.7 a
<i>Calotropis procera</i>	13.2 d	75.1 b	10.5 d	80.3 a
<i>Acacia nilotica</i>	15.7 c	70.4 b	11.6 c	78.3 a
<i>Achillea fragrantissima</i>	23.7 b	55.5 c	20.6 b	59.8 b

^a DS = Disease severity and DR = Disease reduction.

^b Each value represents the mean of three replicates.

^c Values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P = 0.01$).

Table 5. Mean tomato fruit yields from field-grown plants treated with fungicide, or aqueous or ethanol extracts from three medicinal plants.*

Plant extracts	Aqueous extracts		Ethanol extracts	
	Yield (g)	Increase (%)	Yield (g)	Increase (%)
Inoculated and not treated	258.6 ^a e ^b	0.0 e	258.6 d	0.0 d
Mancozeb (0.2%)	493.5 a	90.9 a	493.5 a	90.9 a
<i>Calotropis procera</i>	481.4 b	86.2 b	495.6 a	91.7 a
<i>Acacia nilotica</i>	431.6 c	66.7 c	451.7 b	77.4 b
<i>Achillea fragrantissima</i>	373.7 d	44.5 d	391.3 c	54.4 c

^a Each value represents the mean of three replicates.

^b Values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P = 0.01$).

similar trend to that observed for the reduction of early blight severity.

Discussion

The present study has demonstrated that fungitoxic compounds were found in the three medicinal plants under study, since the plant extracts inhibited growth and conidium germination of *A. solani*. These results are in agreement with other reports where different plant extracts have been assessed (Aslam *et al.*, 2010; Goussous *et al.*, 2010; Suleiman, 2010; Sallam, 2011; Sallam and Abo-Elyousr, 2012; Ravikumar and Garampalli, 2013; Khafari *et al.*, 2014).

The potency of plant extracts differed with the concentration and extractions solvent. Ethanol extracts were more effective than aqueous extracts, indicating that water did not dissolve all the bioactive compounds present in the plants, while these could have been present in the ethanol extracts. Ethanol is an organic solvent and dissolves organic compounds more completely than water, and hence is likely to have liberated the active antifungal phytochemical compounds (Ekwenye and Elegalam, 2005). Stephan *et al.* (2005) suggested that aqueous extracts showed a poorer yield of active compounds than organic solvent extracts, and the fungicidal materials are mostly lipophilic.

The efficacy of medicinal plant extracts may induce a systemic resistance in host plants against pathogens (Lubaina and Murugan, 2013). The extracts may also delay development of infections at early growth stages by inhibiting the mycelial growth of pathogens (Krebs and Forrer, 2001), or the toxic effect of active principles may act directly on the pathogens (Amadioha, 2000).

The extract from *C. procera* was the most effective against fungal growth compared with the other plant extracts. The results confirmed the presence of phytochemicals such as flavonoids, alkaloids and cardiac glycosides in high amounts in *C. procera* as compared with the other two plant species. The aggressive antifungal potential of *C. procera* may be attributed to these phytochemicals. Our results are in accordance with those of Mainasara *et al.* (2012), Tiwari and Singh (2014) and Ajiboso *et al.* (2015). Latex produced by *C. procera* and the members of the Asclepiadaceae contains many bioactive components which can be used for their antimicrobial activity (Nenaah, 2013; Ujwala and Karpagan, 2013).

Many studies have shown effects of active compounds in plant extracts which act as natural fungicides. However, few studies have referred to the action mechanism of these compounds on the microbial cells. For instance, these compounds may increase fungal cell plasma membrane permeability (Imelouane *et al.*, 2009). In addition, these compounds at low concentrations lead to lipid peroxidation in fungi and increase ergosterol biosynthesis (Lucini *et al.*, 2006). Moreover, the toxic effects of phenolic compounds possibly results from denature of fungal proteins (Cowan, 1999). These compounds can also inhibit the protective enzyme activity and subsequently inhibit one or more biochemical pathways (Xing *et al.*, 2012).

In conclusion, this study has revealed the potential of aqueous and ethanol extracts of *A. nilotica*, *A. fragrantissima* and *C. procera* as alternatives to synthetic chemicals for control of early blight of tomato. Since these plants are wild and available, they could be an economic and eco-friendly disease management. With ease of extraction methods, these plant extracts have potential to be harnessed for control this important disease.

Acknowledgments

The authors thank the Faculty of Science, University of Damietta, Egypt for scientific support. The

authors do not have any financial relationship with any organization regarding this research, and have full control of all primary data and agree to allow the full review their data if requested. The experiments performed in this study are compliant with the current laws of Egypt.

Literature cited

- Ajiboso S.O., M.T. Yakubu and A.T. Oladiji, 2015. Chemical profile of aqueous extract of *Calotropis procera* leaf. *World Journal of Pharmaceutical Research* 4, 362–369.
- Akila G., C.R. Einstein A.M. Sathiy, 2012. Treatment of tomato plants with yeast extract glucan induce chitinase activity, resistance to *Alternaria solani*, the causal agent of early blight disease. *International Journal of Biology, Pharmacy and Allied Sciences* 1, 1492–1499.
- Al-Askar A.A. and Y.M. Rashad, 2010. Efficacy of some plant extracts against *Rhizoctonia solani* on pea. *Journal of Plant Protection Research* 50, 239–243.
- Al-Askar A.A., Y.M. Rashad and W.M. Abdulkhair, 2014. Evaluation of the antimicrobial potential of selected medicinal plant extracts against some plant and human pathogens. *Journal of Pure and Applied Microbiology* 8, 159–168.
- Alberto B., A. Zambonelli and A. Zechini, 1997. Ultra structural studies of the effects of *Allium sativum* on Phytopathogenic fungi *in vitro*. *Plant Disease* 81(11), 1241–1246.
- Alsohaili S.A. and A.T. Al-fawwaz, 2014. Composition and antimicrobial activity of *Achillea fragrantissima* essential oil using food model media. *European Scientific Journal* 10, 156–165.
- Amadioha A.C., 2000. Controlling rice blast *in vitro* and *in vivo* with extracts of *Azadirachta indica*. *Crop Protection* 19, 287–290.
- Aslam A., F. Naz, M. Arshad, R. Qureshi and C.A. Rauf, 2010. *In vitro* antifungal activity of selected medicinal plant diffusates against *Alternaria solani*, *Rhizoctonia solani* and *Macrophomina phaseolina*. *Pakistan Journal of Botany* 42, 2911–2919.
- Cohen Y., U. Gisi and E. Mosinger, 1991. Systemic resistance of potato plants against *Phytophthora infestans* induced by unsaturated fatty acid. *Physiological and Molecular Plant Pathology* 38, 255–263.
- Cowan M.M., 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews* 12, 564–568.
- Ekwenye U.N. and N.N. Elegalam, 2005. Antibacterial activity of Ginger (*Zingiber officinale* Roscoe) and garlic (*Allium sativum* L.) extracts on *Escherichia* and *Salmonella typhi*. *International Journal of Molecular Medicine and Advance Sciences* 1, 411–416.
- Glala A.A., A.M. Hoda and Z.F. Fawzi, 2005. Improving tomato plant growth, health, earliness, productivity and fruit quality by chemically induced systematic resistance. *Journal of Applied Sciences Research* 1, 362–372.
- Goussous S.J., F.M. Abu-El-Samen and R.A. Tahhan, 2010. Antifungal activity of several medicinal plants extracts against the early blight pathogen (*Alternaria solani*). *Archives of Phytopathology and Plant Protection* 43, 1746–1758.

- Imelouane B., H. Amhamadi, P. Wathelet, M. Ankit, K. Khedid and A. El-Bachiri, 2009. Chemical composition and antimicrobial activity of essential oil of thyme (*Thymus vulgaris*) from Eastern Morocco. *International Journal of Agriculture and Biology* 11, 205–208.
- Isman B.M., 2000. Plant essential oils for pest and disease management. *Crop Protection* 19, 603–608.
- Khafari A., S. Bahraminejad and S. Abbasi, 2014. Evaluation of the anti-*Alternaria solani* activity of *Allium hirtifolium* Boiss. *Pakistan Journal of Botany* 46, 741–747.
- Krebs H. and H.R. Forrer, 2001. Potatoes: effects of incorporation of medicinal plants into soil. *Agrarforschung Schweiz* 8, 470–475.
- Lubaina A.S. and K. Murugan, 2013. Induced systemic resistance with aqueous extract of *Mikania scandens* (L.) Wild. against *Alternaria sesame* (Kawamura) Mohanty and Behera in *Sesamum orientale* L. *Journal of Crop Science and Biotechnology* 16, 269–276.
- Lucini E.I., M.P. Zunino, M.I. Lopez and J.A. Zygadlo, 2006. Effect of monoterpenes on lipid composition and sclerotial development of *Sclerotium cepivorum* Berk. *Journal of Phytopathology* 154, 441–446.
- Mainasara M.M., B.L. Aliero, A.A. Aliero and M. Yakubu, 2012. Phytochemical and antibacterial properties of root and leaf extracts of *Calotropis procera*, *Nigerian Journal of Basic and Applied Sciences* 20, 1–6.
- Maya C. and M. Thippanna, 2013. *In vitro* evaluation of ethano-botanically important plant extracts against early blight disease (*Alternaria solani*) of tomato. *Global Journal of Bioscience and Biotechnology* 2, 248–252.
- Mir M.A., S.S. Sawhney and M.M.S. Jassal, 2013. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. *Wudpecker Journal of Pharmacy and Pharmacology* 2, 1–5.
- Nair K.R.S. and A.H. Ilingboe, 1962. Method of controlled inoculations with conidiophores of *Erysiphe graminis* var. *tritici*. *Phytopathology* 52, 714.
- Nenaah G., 2013. Antimicrobial activity of *Calotropis procera* Ait. (Asclepiadaceae) and isolation of four flavonoid glycosides as the active constituents. *World Journal of Microbiology and Biotechnology* 29, 1255–1262.
- Nenaah E.G. and M.E. Ahmed, 2011. Antimicrobial activity of extracts and latex of *Calotropis procera* (Ait.) and synergistic effect with reference antimicrobials. *Research Journal of Medicinal Plants* 5, 706–716.
- Ravikumar M.C. and R.H. Garampalli, 2013. Antifungal activity of plants extracts against *Alternaria solani*, the causal agent of early blight of tomato. *Archives of Phytopathology and Plant Protection* 46, 1897–1903.
- Rivillas-Acevedo L. and M. Soriano-García, 2007. Antifungal activity of a protean extract from *Amaranthus hypochondriacus* seeds. *Journal of the Mexican Chemical Society* 51, 136–140.
- Saleh N.A.M., 2003. Global phytochemistry: The Egyptian experience. *Phytochemistry* 63, 239–241.
- Sallam M.A.N. and K.A.M. Abo-Elyousr, 2012. Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. *Plant Protection Science* 49, 74–79.
- Sallam N.A., 2011. Control of tomato early blight disease by certain aqueous plant extracts. *The Plant Pathology Journal* 10, 187–191.
- Satish S., D.C. Mohana and M.P. Raghvendra, 2007. Antifungal activity of some plant extracts against important seed-borne pathogens of *Aspergillus* sp. *Journal of Agricultural Technology* 3, 109–119.
- Schmitz H., 1930. Poisoned Food Technique. *Industrial and Engineering Chemistry-Analytical Edition* 2, 361–363.
- Shafik A. and W. Elseesy, 2003. Medicine in ancient Egypt. In: *Medicine Across Cultures* (H. Seli, H. Shapiro, eds.), Boston, Kluwer, 27–48.
- Stephan G., A. Schmitt, S.M. Corvalho, B. Seddon and E. Koch, 2005. Evaluation of biocontrol preparations and plant extracts for the control of *Phytophthora infestans* on potato leaves. *European Journal of Plant Pathology* 112, 235–346.
- Suleiman M.N., 2010. Fungitoxic activity of neem and paw-paw leaves extracts on *Alternaria solani*, causal organism of yam rots. *Advances in Environmental Biology* 4, 159–161.
- Tiwari A.S. and S. Singh, 2014. Chemical analysis of leaf extracts of *Calotropis procera*. *International Journals of Scientific Research Publications* 4, 1–2.
- Ujwala K. and N. Karpagan, 2013. Potential therapeutical values of plant lattices. *International Journal of Medicinal and Aromatic Plants* 3, 317–325.
- Xing Y., Q. Xu, X. Li, Z. Che and J. Yun, 2012. Antifungal activities of clove against *Rhizopus nigricans*, *Aspergillus flavus* and *Penicillium citrinum* *in vitro* and in wounded fruit test. *Journal of Food Safety* 32, 84–93.

Accepted for publication: December 28, 2015

Published online: May 14, 2016