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Antifungal Potential and Biochemical Effects of Monoterpenes and Phenylpropenes on Plant Pathogenic Fungi

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Abstract

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To develop new natural fungicides, six monoterpenes and two phenylpropenes were tested for their antifungal activity against eight plant pathogenic fungi. The results of the mycelial growth inhibition assay showed that *trans*-cinnamaldehyde was the most potent compound against the eight tested fungi with EC_{50} values ranging between 0.75 and 3.19 mg/l. This compound caused the higher mycelial growth inhibition than carbendazim. Furthermore, (–)-menthone exhibited strong antifungal activity against *Alternaria solani* (EC_{50} = 9.31 mg/l), *Penicillium digitatum* (EC_{50} = 16.14 mg/l), and *Rhizoctonia solani* (EC_{50} = 24.69 mg/l). Likewise, eugenol showed potent antifungal activity against *P. digitatum*, *R. solani*, *Fusarium solani*, and *A. solani*, whereas EC_{50} values were less than 30.0 mg/l. In a separate experiment, *trans*-cinnamaldehyde, *p*-cymene, eugenol, and (–)-menthone were evaluated for their inhibitory effects on pectin methyl esterase and cellulase. The tested compounds exhibited the pronounced inhibition of enzyme activities with *trans*-cinnamaldehyde being the most potent inhibitor for both enzymes.

Keywords: natural products; antifungal activity; enzyme inhibition

Monoterpenes are a class of plant secondary metabolites containing ten carbons. These compounds are the main constituents of essential oils that give plants their unique odoriferous properties because of their low boiling points. They are derived from the coupling of two isoprenoid units, which are made from isopentylpyrophosphate, a precursor in the biosynthesis of cholesterol (Tsao & Coats 1995). Monoterpenes have the potential to be used in plant protection because of their unique properties, such as lipophilicity, low vapour pressure, and low mammalian toxicity. Several biological activities of monoterpenes were described, including insecticidal, herbicidal, fungicidal, and bactericidal properties (Duke *et al.* 2000; Grodnitzky & Coats 2002; Wuryatmo *et al.* 2003; Cantore *et al.* 2009).

Phenylpropenes are a subfamily of compounds under phenylpropanoids that are synthesised in plants using phenylalanine. They are the second largest group of plant volatiles and are among the major components of plant derived essential oils (Benzoukian 1986). Phenylpropenes were reported to possess a wide spectrum of biological activity including the antimicrobial one (Harborne & Baxter 1993; Cheng *et al.* 2008).

The antifungal activity of some monoterpenes and phenylpropenes against plant pathogenic fungi has been documented. For example, the antifungal activity of 22 monoterpenes and phenylpropenes was evaluated against two postharvest pathogens *Botrytis cinerea* and *Monilinia fructicola*. Among the compounds, carvacrol and thymol revealed the

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highest fungicidal activity (Tsao & Zhou 2000). In addition, the antifungal activities of oxygenated monoterpenes were examined against 31 plant pathogenic fungi (Kordali *et al.* 2007). Some of the examined monoterpenes showed mycelial growth inhibitory effects against certain tested fungal species. It has been reported that thymol completely inhibited the mycelial growth of 17 phytopathogenic fungi, including *Rhizoctonia solani* and *Fusarium oxysporum* (Kordali *et al.* 2008). Carvone had a potential to control potato sprouting and it had promising antifungal activity against other potato storage diseases caused by *F. sulphureum, Phoma exigua* var. *foveata*, and *Helminthosporium solani* (Hartmans *et al.* 1995). Similarly, Garcia *et al.* (2008) demonstrated that L-carvone strongly inhibited the growth of post-harvest fungi *Colletotrichum musae*, *C. gloeosporioides*, and *F. subglutinans* f.sp. *ananas*. Furthermore, geranial was reported to possess fungistatic and fungicidal effects against *Penicillium digitatum*, *P. italicum*, and *Geotrichum candidum* (Wuryatmo *et al.* 2003).

In a recent study, we have examined the antifungal activity and possible modes of action of twelve monoterpenes against four plant pathogenic fungi (Marei *et al.* 2012). Among the examined monoterpenes, thymol and (*S*)-limonene revealed promising antifungal activity. In the present study, six monoterpenes and two phenylpropenes were evaluated for their antifungal activity against eight plant pathogenic fungi *Aspergillus niger*, *Alternaria solani*, *Botrytis cinerea*, *F. oxysporum*, *F. solani*, *P. digitatum*, *Phytophthora infestans*, and *R. solani*. In addition, the inhibitory effect of monoterpenes on pectin methyl esterase and cellulase activities was also examined to explore mechanisms of their antifungal action.

MATERIAL AND METHODS

Monoterpenes and phenylpropenes. The monoterpenes, (–)-citronellal (95%), *p*-cymene (99%), (–)-menthone (90%), α-pinene (98%), α-terpinene (85%), and (–)-terpinen-4-ol (95%), and the phenylpropenes, *trans*-cinnamaldehyde (99%), and eugenol (99%) (Figure 1), were purchased from Sigma Aldrich Chemical Co. (Steinheim, Germany). Carbendazim (95%) was supplied by Kafr El-Zayat Pesticides and Chemicals Co. (Gharbia Governorate, Egypt) and used as a reference fungicide. All chemicals were of the highest grade commercially available.

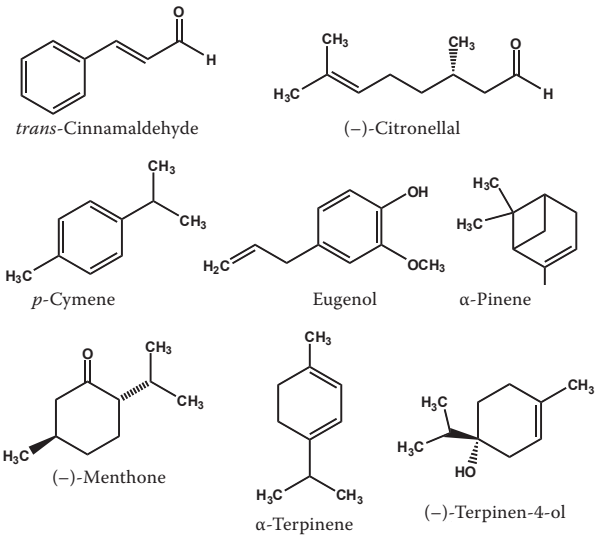

Figure 1. Chemical structure of monoterpenes and phenylpropenes

Fungal strains. Eight plant pathogenic fungal species were obtained from the Fungicide Bioassay Laboratory, Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University. The fungal species used in the experiments were *Aspergillus niger* (Tiegh.) isolated from *Solanum melogena*, *Alternaria solani* Sorauer (Ellis) isolated from leaves of *Solanum tuberosum*, *Botrytis cinerea* (Persoon) isolated from *Fragaria ananassa*, *Fusarium oxysporum* (Schltdl.) isolated from *Zea mays* seeds, *Fusarium solani* (Mart.) Sacc. isolated from tubers of *Solanum tuberosum*, *Penicillium digitatum* (Pers.) isolated from *Citrus sinensis* fruits, *Phytophthora infestans* (Mot.) isolated from leaves of *Solanum tuberosum*, and *Rhizoctonia solani* (Kuhn.) isolated from *Phaseolus vulgaris*. The fungal cultures were maintained on a potato dextrose agar medium (PDA): potato 200 g, dextrose 20 g, and agar 15 g in 1 l of distilled water at 25°C.

Antifungal assay. The antifungal activity of the test monoterpenes and phenylpropenes was determined using a mycelial radial growth inhibition technique (Zambonelli *et al.* 1996; Bajpai *et al.* 2007). Appropriate volumes of the stock solutions of monoterpenes and phenylpropenes in dimethylsulphoxide (DMSO) were added to the PDA medium immediately before it was poured into Petri dishes (9.0 cm diameter) at 40–45°C. The tested compounds and a reference fungicide (carbendazim) were evaluated at concentrations of 1, 2.5, 5, 10, 25, 50, 100, 250, and 500 mg/l. *trans*-Cinnamaldehyde was tested at concentrations of 0.5, 1, 2, 4, 8, and 10 mg/l. Each concentration was

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tested in triplicate. Parallel controls were maintained with DMSO mixed with PDA. The 5 mm diameter discs of mycelial hyphae were taken from 8-day-old cultures on PDA plates and placed upside down in the centre of the Petri dishes. The plates were incubated at 27°C in the dark. Colony growth diameter was measured after the fungal growth in the control treatments had reached the edges of the plates. Growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control. The percentage of mycelial growth inhibition was calculated from the formula (Pandey *et al.* 1982):

$$\text{Mycelial growth inhibition} = [(DC - DT)/DC] \times 100$$

where: DC, DT – average diameter (mm) of the fungal colony of the control and the treatment, respectively.

The concentration of the compound that inhibits the mycelial growth of fungi by 50% (EC_{50}) was determined by a linear regression method (Finney 1971).

Pectin methyl esterase (PME) activity assay. The tested fungi were grown on a potato dextrose (PD) medium supplied with 1% pectin (apple pectin apipectin 150 SAG). The pH of the medium was adjusted to 7.0 by using 0.05 N sodium hydroxide solution and autoclaved for 15 minutes. The autoclaved medium was readjusted to pH 7.0 with the sterile 0.05 N sodium hydroxide and then inoculated with fungi. After 8 days of incubation at 27°C, the medium was filtrated through Whatman No. 1 paper. The filtrate was used as a source of the crude pectin methyl esterase enzyme. The activity of PME was measured according to the method described by Talboys and Busch (1970) with some modifications. To 7 ml of the reaction mixture (pectin 0.5 g, sodium chloride 0.58 g, bromothymol blue solution 0.05% (2.5 ml), chloroform 4 ml, and distilled water up to 1000 ml (pH 7), 2.0 ml of the crude enzyme and 1 ml of the tested compound were added. The compounds were tested at final concentrations of 0.5, 1, 2.5, 5, 10, 25, 50, 100, 200, 300, 400, and 500 mg/l. The treatments were incubated at 30°C for 24 h, and then titrated to pH 7.0 with sodium hydroxide (0.01 N). Control (without test compound) and blank (without crude enzyme) were prepared. Each treatment was replicated three times. The inhibition percentage of the PME activity was calculated from the equation:

$$I\ (\%) = [(A - B)/A] \times 100$$

where: A – volume (ml) of NaOH (0.01 N) in control treatment; B – volume (ml) of NaOH (0.01 N) in treatment

IC_{50} (concentration of the compound required to cause a 50% inhibition of enzymatic activity) values were determined by a linear regression method (Finney 1971).

Cellulase activity assay. Fungal cultures were grown on a potato dextrose (PD) medium amended with 3% of carboxymethyl cellulose for 12 days at 27°C. The medium was filtrated through Whatman No. 1 paper. The filtrate was used as a source of the crude cellulase enzyme. The crude enzyme (1 ml) was added to citrate buffer, pH 4.8 (2 ml) and the mixture was warmed in a water bath at 50°C for 30 minutes. Then 1 ml of the tested compounds was added and incubated at 28°C for 24 hours. The monoterpenes and phenylpropenes were tested at concentrations of 0.5, 1, 2.5, 5, 10, 25, 50, 100, 200, 300, 400, and 500 mg/l. Then 3 ml of the reaction mixture [3,5-dinitrosalicylic acid (10 g), sodium hydroxide (10 g), phenol (20 ml), sodium sulphate (0.5 g), and distilled water up to 1000 ml] was added. Three replicates of each treatment, control and blank (without enzyme) were prepared. After incubation for 15 min at 50°C in a water bath, the absorbance was measured at 575 nm. The inhibition percentage of cellulase activity was calculated from the equation:

$$I\ (\%) = [(Ac - At)/Ac] \times 100$$

where: Ac – absorbance in control; At – absorbance in treatment

Statistical analysis. Statistical analysis was performed using the SPSS v21.0 software program (Chicago, USA). The concentration-response data were subjected to Probit analysis to obtain the EC_{50} and IC_{50} values (Finney 1971). The values of EC_{50} and IC_{50} were considered to be significantly different if the 95% confidence limits did not overlap.

RESULTS

Antifungal activity of monoterpenes and phenylpropenes. The inhibitory effects of six monoterpenes and two phenylpropenes on the mycelial growth of eight phytopathogenic fungal species are shown in Table 1. All of the test monoterpenes caused mycelial growth inhibition. However, the antifungal potency was dependent on the specific compound and fungal species tested. *trans*-Cinnamaldehyde exhibited the highest antifungal activity against the eight fungal species. The EC_{50} values of this compound ranged between 0.75 and 3.19 mg/l. The results showed that some of the tested compounds revealed promising antifungal activity as their

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Table 1. Comparative antifungal activity of monoterpenes and phenylpropenes against plant pathogenic fungi

Monoterpene	EC_{50} (mg/l) (95% confidence limits)							
	A. niger	*A. solani*	*B. cinerea*	*F. oxysporum*	*F. solani*	*P. digitatum*	*P. infestans*	*R. solani*
trans-Cinnamaldehyde	3.19 (2.17–6.93)	2.44 (2.12–2.85)	1.42 (1.22–1.63)	1.56 (1.28–1.87)	1.31 (0.76–1.96)	0.75 (0.59–0.90)	1.80 (1.05–3.00)	2.57 (1.70–4.33)
(–)-Citronellol	240.7 (152.0–339.8)	54.35 (29.64–89.22)	234.3 (121.7–614.9)	303.7 (286.6–337.3)	138.8 (84.7–195.4)	67.23 (46.09–94.90)	63.43 (5.73–168.8)	202.7 (173.9–225.2)
p-Cymene	63.52 (21.50–124.9)	195.1 (84.7–291.7)	130.2 (48.71–183.6)	177.9 (109.2–235.1)	73.84 (22.20–160.8)	48.05 (40.39–56.15)	144.7 (44.57–317.8)	53.23 (5.85–146.0)
Eugenol	76.55 (47.63–122.7)	36.37 (19.0–63.45)	59.45 (33.71–92.87)	53.82 (43.39–64.98)	35.43 (11.02–112.6)	16.14 (13.71–18.92)	68.19 (36.50–95.47)	25.95 (7.89–57.17)
(–)-Menthone	157.9 (45.81–433.0)	9.31 (6.13–12.90)	47.82 (25.88–74.46)	248.2 (33.44–340.6)	254.3 (132.1–419.6)	43.54 (25.85–63.58)	16.42 (3.78–35.37)	24.69 (19.24–30.89)
α-Pinene	102.3 (63.6–162.4	78.64 (35.83–156.4)	100.3 (67.92–146.5)	75.41 (46.56–122.1)	120.5 (65.14–229.8)	128.5 (50.02–399.6)	111.8 (67.89–180.9)	41.05 (20.18–70.10)
α-Terpinene	132.6 (88.36–177.8)	187.8 (129.5–161.9)	77.91 (52.46–112.3)	218.7 (183.7–261.9)	96.27 (69.49–131.4)	98.41 (41.02–192.2)	71.53 (58.08–87.11)	227.1 (162.7–293.5)
(–)-Terpinen-4-ol	187.7 (101.1–320.7)	54.35 (29.64–89.22)	118.1 (74.0–171.4)	241.0 (196.2–321.0)	213.6 (157.4–268.4)	71.4 (28.3–156.3)	36.11 (26.11–46.86)	325.3 (280.6–387.6)
Carbendazim	18.61 (13.92–25.38)	11.01 (6.45 –15.26)	46.35 (24.92–141.0)	37.98 (27.73–55.59)	28.8 (11.64–64.22)	13.63 (10.61–17.53)	13.12 (10.04–16.03)	25.14 (13.04–60.47)

EC_{50} values were less than 100 mg/1. For example, *p*-cymene (EC_{50} = 63.62 mg/l) and eugenol (EC_{50} = 76.55 mg/l) showed strong antifungal activity against *A. niger*, while (–)-citronellol was the least effective. Similarly, (–)-menthone (EC_{50} = 9.31 mg/l), eugenol (EC_{50} = 36.37 mg/l), (–)-citronellol (EC_{50} = 54.35 mg/l), (–)-terpinene-4-ol (EC_{50} = 54.35 mg/l), and α-pinene (EC_{50} = 78.64 mg/l) exhibited potent antifungal activity against *A. solani*. In the case of *B. cinerea*, (–)-menthone, eugenol, and α-terpinene were among the most potent mycelial growth inhibitors. In contrast, (–)-citronellol caused the weakest mycelial growth inhibition against this fungus. Eugenol (EC_{50} = 53.82 mg/l) and α-pinene (EC_{50} = 75.41 mg/l) had strong antifungal activity against *F. oxysporum*, while (–)-citronellol was the least effective. Among the tested compounds, eugenol, *p*-cymene, and α-terpinene were the most effective inhibitors of *F. solani* mycelial growth, whereas (–)-menthone was the less effective one. All of the tested compounds showed strong antifungal activity against *P. digitatum* except α-pinene (EC_{50} = 128.5 mg/l). Similarly, the tested monoterpenes were highly effective against *P. infestans* except *p*-cymene (EC_{50} = 144.7 mg/l) and α-pinene (EC_{50} = 111.8 mg/l). In the case of *R. solani*, (–)-menthone, eugenol, a-pinene and *p*-cymene revealed the higher antifungal activity than (–)-terpinene-4-ol, a-terpinene and (–)-citronellol.

The fungal species tested in this study exhibited different sensitivities to monoterpenes and phenylpropenes (Table 1). For example, *P. digitatum* was the most sensitive fungus to *trans*-cinnamaldehyde, *p*-cymene, and eugenol. Moreover, *A. solani* was the most sensitive fungus to (–)-citronellol and (–)-menthone. In addition, *R. solani* and *P. infestans* were the most sensitive fungi to α-pinene and α-terpinene, respectively. In contrast, *A. niger* was the least sensitive fungus to *trans*-cinnamaldehyde and eugenol. Similarly, *F. oxysporum* was the less sensitive fungus to (–)-citronellol and *p*-cymene. Moreover, *R. solani* was the least sensitive fungus to α-terpinene and (–)-terpinene-4-ol. In addition, *F. solani* and *P. digitatum* were the least sensitive fungi to (–)-menthone and α-pinene, respectively.

Among the compounds studied, *trans*-cinnamaldehyde showed antifungal activity higher than carbendazim (reference fungicide) against the eight fungi. Likewise, (–)-menthone was more toxic than carbendazim to *A. solani* and *R. solani*. Eugenol had antifungal activity comparable to carbendazim against *R. solani* and *P. digitatum*.

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Table 2. Inhibitory effects of monoterpenes and phenylpropenes on pectin methyl esterase and cellulase activities

Monoterpene	Fungus	IC_{50} (mg/l) (95% confidence limits)[a]	
		pectin methyl esterase	cellulase
trans-Cinnamaldehyde	*A. niger*	3.70 (3.02–4.74)	1.92 (1.60–2.29)
	A. solani	2.08 (1.86–2.43)	2.17 (1.85–2.54)
	B. cinerea	2.49 (2.13–2.94)	3.13 (2.08–5.42)
	F. oxysporum	1.88 (1.58–2.24)	3.30 (2.74–4.09)
	F. solani	3.72 (2.98–4.89)	3.75 (3.16–4.57)
	P. digitatum	3.23 (2.77–3.83)	3.66 (3.04–4.56)
	P. infestans	2.14 (1.77–2.59)	1.80 (1.56–2.06)
	R. solani	3.74 (2.72–4.69)	1.74 (1.04–2.75)
p-Cymene	*A. niger*	> 500	13.58 (1.54–45.77)
	A. solani	39.63 (18.71–76.21)	108.9 (49.43–242.6)
	B. cinerea	74.89 (34.98–157.3)	129.3 (68.05–254.7)
	F. oxysporum	203.9 (127.1–320.8)	214.6 (106.0–527.9)
	F. solani	78.50 (32.76–186.5)	473.9 (227.2–1407.2)
	P. digitatum	71.45 (30.43–160.8)	301.3 (130.9– 1568.8)
	P. infestans	43.38 (23.67–74.85)	5.80 (1.15–15.37)
	R. solani	121.0 (50.49–320.0)	105.2 (95.0–246.8)
Eugenol	*A. niger*	35.61 (17.46–65.94)	44.34 (16.68–102.4)
	A. solani	48.86 (22.48–97.99)	116.7 (54.37–262.1)
	B. cinerea	47.14 (33.35–65.36)	256.4 (111.6–770.0)
	F. oxysporum	64.78 (43.99–94.44)	76.05 (55.63–103.4)
	F. solani	231.1 (108.5–607.6)	245.1(106.8–786.1)
	P. digitatum	349.5 (198.4–710.3)	34.27 (13.79–73.03)
	P. infestans	46.29 (22.38–89.01)	30.07 (22.01–40.19)
	R. solani	> 500	60.76 (25.60–135.1)
(–)-Menthone	*A. niger*	142.4 (74.19–293.3)	57.30 (33.59–94.87)
	A. solani	279.4 (150.2–644.4)	53.32 (20.83–124.6)
	B. cinerea	19.99 (12.91–29.36)	99.74 (42.76–238.6)
	F. oxysporum	29.36 (7.17–82.03)	58.76 (29.37–111.3)
	F. solani	355.9 (294.3–425.1)	195.4 (87.10–538.8)
	P. digitatum	441.6 (249.0–953.0)	27.46 (12.80–52.32)
	P. infestans	15.93 (0.74–69.42)	69.41 (45.52–103.6)
	R. solani	306.2 (141.1–871.2)	58.11 (29.39–109.5)
Carbendazim	*A. niger*	> 500	11.57 (6.74–18.23)
	A. solani	3.28 (1.39–6.06)	14.39 (8.20–23.18)
	B. cinerea	19.27 (12.77–27.92)	114.3 (48.67–456.1)
	F. oxysporum	11.50 (0.28–57.58)	70.66 (26.99–202.5)
	F. solani	8.48 (3.10–17.95)	44.11 (9.23–191.6)
	P. digitatum	18.81 (10.79–30.48)	110.0 (56.84–279.9)
	P. infestans	7.42 (4.04–12.24)	24.08 (2.09–123.1)
	R. solani	28.27 (3.03–139.3)	32.30 (12.74–18.23)

[a]concentration causing 50% enzyme inhibition

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Effect of monoterpenes and phenylpropenes on pectin methyl esterase and cellulase activity. The most potent antifungal compounds, *trans*-cinnamaldehyde, *p*-cymene, eugenol, and (–)-menthone, were tested for their inhibitory effects on the activity of pectin methyl esterase. This enzyme is among common target enzymes of conventional fungicides. *trans*-Cinnamaldehyde (IC_{50} values ranged between 1.88 and 3.74 mg/l) showed the highest inhibition of pectin methyl esterase activity, followed by carbendazim (Table 2). The enzyme isolated from eight fungi was highly sensitive to *trans*-cinnamaldehyde. However, *trans*-cinnamaldehyde showed higher inhibitory activity on the enzyme isolated from *F. oxysporum*, *A. solani*, *P. infestans*, and *B. cinerea* than the enzyme isolated form *A. niger*, *F. solani*, *P. digitatum*, and *R. solani*. *p*-Cymene caused variable inhibitory effects on the enzyme isolated from the tested fungi. This compound revealed the highest inhibitory activity on pectin methyl esterase isolated from *A. solani* (IC_{50} = 39.63 mg/l) and *P. infestans* (IC_{50} = 43.38 mg/l), but it was not active on the enzyme isolated from *A. niger* (IC_{50} > 500 mg/l). Eugenol caused strong inhibition of the enzyme isolated from the tested fungi except that isolated from *P. digitatum* and *F. solani*. On the contrary, eugenol showed no inhibitory effect on the enzyme isolated from *R. solani* (IC_{50} > 500 mg/l). In the case of (–)-menthone, strong inhibition of the enzyme isolated from *P. infestans* (IC_{50} = 15.93 mg/l), *B. cinerea* (IC_{50} = 19.99 mg/l), and *F. oxysporum* (IC_{50} = 29.36 mg/l), and weak inhibition of the enzyme isolated from other fungi were observed.

On the other hand, the inhibitory effect of *trans*-cinnamaldehyde, *p*-cymene, eugenol, and (–)-menthone on the activity of cellulase isolated from eight fungal species is presented in Table 2. Based on the concentration causing 50% enzyme inhibition (IC_{50} values), *trans*-cinnamaldehyde was the most potent inhibitor for cellulase isolated from the eight tested fungi. The IC_{50} values of *trans*-cinnamaldehyde ranged between 1.80 and 3.75 mg/l. *trans*-Cinnamaldehyde also caused higher enzyme inhibition than carbendazim (IC_{50} values ranged between 11.57 and 114.3 mg/l). In addition, *p*-cymene displayed strong inhibition of cellulase isolated from *P. infestans* and *A. niger*, and weak inhibitory effect on cellulase isolated from the other fungi. (–)-Menthone exhibited a pronounced inhibitory effect on cellulase isolated from the fungal species except *R. solani*. The cellulase isolated from *F. oxysporum*, *A. solani*, *P. infestans*, *A. niger*, and *P. digitatum* was strongly inhibited by eugenol. In contrast, eugenol caused a relatively weak inhibitory effect on cellulase isolated from *R. solani*, *B. cinerea*, and *F. solani*. Although (–)-menthone and eugenol revealed the lower inhibition of cellulase isolated from the eight tested fungi than *trans*-cinnamaldehyde, they were more effective than carbendazim in the inhibition of cellulase.

DISCUSSION

The antifungal activity of some tested monoterpenes and phenylpropenes has been previously reported against other plant pathogenic and food spoilage fungi. For example, *trans*-cinnamaldehyde and eugenol were reported to possess antifungal activity against the white rot fungus *Coriolus versicolor* and the brown rot fungus *Laetiporus sulphureus* and *trans*-cinnamaldehyde was more effective on *C. versicolor* than eugenol (Wang *et al.* 2005). These findings supported our results in which *trans*-cinnamaldehyde was more effective than eugenol against the tested fungi. Similarly, strong antifungal activity of *trans*-cinnamaldehyde against *F. oxysporum* f.sp. *gladioli* was demonstrated by Barrera-Necha *et al.* (2009). Also, it has been stated that (–)-citronellol and (–)-terpinen-4-ol caused the mycelial growth inhibition of food spoilage fungi *Aspergillus*, *Penicillium*, and *Fusarium* species (Aoudou *et al.* 2010). Furthermore, (–)-menthone was found to cause the higher mycelial growth inhibition of *B. cinerea* than *p*-cymene and α-pinene (Bouchra *et al.* 2003). These results are in agreement with data obtained from antifungal activity of these three monoterpenes. Terpinene-4-ol and eugenol were shown to possess antifungal activity against *F. oxysporum* (Campaniello *et al.* 2010; Morcia *et al.* 2012). In addition, the antifungal potential of other monoterpenes and phenylpropenes against plant pathogenic fungi was reported (Wuryatmo *et al.* 2003; El-Zemity & Ahmed 2005; Kordali *et al.* 2007, 2008; Zhao *et al.* 2011).

The inhibitory effects of the tested compounds on pectin methyl esterase and cellulase were not previously reported. However, in our earlier study, monoterpenes, such as thymol and (*S*)-limonene, caused a strong inhibitory effect on the activity of these two enzymes isolated from plant pathogenic fungi (Marei *et al.* 2012). The results of the present study showed that some of the tested compounds, such as *trans*-cinnamaldehyde and (–)-menthone,

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caused potent inhibition of pectin methyl esterase and cellulase and, at the same time, revealed strong antifungal activity against the tested fungal species. These findings indicate that the tested compounds may get their antifungal activity through the inhibition of pectin methyl esterase and cellulase. In addition, previous studies demonstrated that some monoterpenes preformed their antifungal action at the membrane level or membrane embedded enzymes (Uribe *et al.* 1985; Sikkema *et al.* 1994). The change in the fatty acid composition of the cell membrane, inhibition of respiration, and alteration in permeability were proposed as possible mechanisms for antifungal effects of monoterpenes (Cox *et al.* 2000; Prashar *et al.* 2003). These findings supported our results which indicated that *trans*-cinnamaldehyde and other tested compounds are potent inhibitors of pectin methyl esterase which modifies the degree of methylesterification of pectin that is the major component of fungal cell walls. Such changes in pectin structure are associated with changes in cellular adhesion, plasticity, pH, and ionic contents of the cell wall and influence fungi development, membrane integrity and permeability. Based on these findings, it can be suggested that the tested monoterpenes and phenylpropenes gain their antifungal activity by inhibiting pectin methyl esterase and cellulase as well as previously reported mechanisms.

The results of antifungal assays showed that *trans*-cinnamaldehyde, an aldehyde, was the most active compound against all tested fungi. In addition, eugenol (an alcohol) and (–)-menthone (a ketone) were among the other potent antifungal compounds. Similar observations were demonstrated by other researchers (Bouchra *et al.* 2003; Wang *et al.* 2005). In general, it has been documented that oxygenated monoterpenes are more biologically active than non-oxygenated ones. Also, the oxygenated monoterpenes with carbonyl group (ketones and aldehydes) and hydroxyl group (alcohols) are more active than oxygenated monoterpenes containing other functional groups (Vokou *et al.* 2003; De Martino *et al.* 2010).

The development of effective natural fungicides would help to decrease the negative impact of synthetic compounds, such as food contamination with fungicide residues, fungal strain resistance, and environmental pollution. In this regard, natural compounds may be used as safe alternatives for management of plant diseases caused by plant pathogenic fungi. In this study, *trans*-cinnamaldehyde, eugenol, and (–)-menthone revealed promising antifungal activity against eight plant pathogenic fungi, particularly *trans*-cinnamaldehyde which was more active than a reference fungicide, carbendazim. Based on the present results, these three compounds could be used as alternative fungicides. However, further studies on formulation, safety, and phytotoxicity of these compounds are needed before field application.

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