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In Vitro and *In Vivo* Inhibition of Plant Pathogenic Fungi by Essential Oil and Extracts of *Magnolia liliflora* Desr.

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ABSTRACT

This study was carried out to evaluate the *in vitro* and *in vivo* antifungal efficacy of essential oil and extracts derived from the flower and leaves of *Magnolia liliflora*, respectively, against plant pathogenic fungi. The oil (750 µg disc^{-1}) and various leaf extracts such as hexane, chloroform, ethyl acetate and methanol (1,500 µg disc^{-1}) revealed promising antifungal effects against *Botrytis cinerea* KACC 40573, *Colletotrichum capsici* KACC 40978, *Fusarium oxysporum* KACC 41083, *Fusarium solani* KACC 41092, *Phytophthora capsici* KACC 40157, *Rhizoctonia solani* KACC 40111 and *Sclerotinia sclerotiorum* KACC 41065 as radial growth inhibition percentages of 38 to 65.6% and 7.6 to 57.3%, respectively along with their respective MIC and MFC values ranging from (125 to 500 and 125 to 100 µg mL^{-1}) and (500 to 4,000 and 500 to 8,000 µg mL^{-1}). The oil had a strong detrimental effect on spore germination of all tested plant pathogens as well as concentration and time-dependent kinetic inhibition of *P. capsici* KACC40157. Also the oil displayed potent *in vivo* antifungal effect against one of the selected plant pathogens *P. capsici* KACC 40157 on greenhouse-grown pepper plants. The results of this study indicate that the flower oil and leaf extracts of *M. liliflora* could be used as natural alternatives to synthetic fungicides to control the *in vitro* and *in vivo* growth of certain important plant pathogenic fungi.

Keywords: Antifungal activity, Essential oil, Leaf extracts, *Magnolia liliflora*, Plant pathogenic fungi.

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INTRODUCTION

Plants are constantly exposed and threatened by a variety of pathogenic microorganisms present in their environments. Diseases caused by plant pathogenic fungi significantly contribute to the overall loss in crop yield worldwide (Savary *et al*., 2006; Montesinos, 2007). In an effort to combat diseases, plants have devised various mechanisms and compounds to fend off microbial invaders. However, despite the existence of defense mechanisms, plants are exposed to attack by plant pathogenic fungi.

Widespread use of pesticides has significant drawbacks including cost, handling hazards, pesticide residues, and threats to human health and environment (Paster and Bullerman, 1988; Arcury *et al*., 2002). For many years, a variety of different synthetic chemicals has been used as antifungal agents to inhibit the growth of plant pathogenic fungi. However, there are series of problems for the effective use of these chemicals in areas where the fungi have developed resistance (Brent and Hollomon, 1998; Schillberg *et al*., 2001). Thus, there is a growing interest on the research on the possible use of natural products such as plant-based essential oils and extracts, which may be less damaging for pest and disease control. Plants have long been recognized to provide a potential source of chemical compounds or more commonly products, known as phytochemicals, which include essential oil

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and plant extracts. Research into plant derived fungicides for their possible applications to control plant pathogenic fungi is being intensified as these have enormous potential to inspire and influence modern agrochemical research. There is a good reason to suppose that the secondary metabolism of plants has evolved to protect them against attack by microbial pathogens (Benner *et al*., 1993). Essential oils and plant extracts may be alternative strategies to the currently used fungicides to control plant pathogenic fungi, because they virtually constitute a rich source of bioactive substances which could lead to the development of new classes of possibly safer disease control agents. Several plant-derived essential oils and extracts have been shown to exert potential antifungal activity against plant pathogenic fungi (Al-Reza *et al*., 2010; Veloz-García *et al*., 2010).

Magnolia liliflora Desr. (Magnoliaceae) is a 3-4 meter high deciduous shrub propagated and distributed in many parts of East Asia and North America. It is widely used in traditional medicine (Duke and Ayensu, 1985). Previously we reported the chemical composition of the essential oil of the flowers of *M. liliflora* and the antibacterial activity of the essential oil and leaf extracts (Bajpai *et al*., 2008). However, antifungal activity of *M. liliflora* against plant pathogenic fungi has not been reported yet.

Therefore, the objective of the present study was to evaluate the *in vitro* and *in vivo* antifungal fungal activities of essential oil and extracts derived from the floral parts and leaves of *M. liliflora*, respectively against certain important plant pathogenic fungi.

MATERIALS AND METHODS

Plant Materials, Isolation of Essential Oil, and Preparation of Leaf Extracts

Collection of the samples, the flowers and leaves of *M. liliflora* for the isolation of the essential oil and various extracts, respectively was carried out as described previously (Bajpai *et al*., 2008).

Chemical and Reagents

The analytical grade solvents (hexane, chloroform, ethyl acetate and methanol) used in this study were purchased from OCI Company Ltd., Seoul, Republic of Korea and had over 99.5% purity. Dimethylsulfoxide (DMSO) and Tween-20 chemicals were purchased from Sigma-Aldrich, St. Louis, MO, USA. Lactophenol-cotton blue was purchased from Hardy Diagnostics, CA, USA.

Plant Pathogenic Fungi

The reference strains of plant pathogenic fungi used in the experiments were *Botrytis cinerea* KACC 40573, *Colletotrichum capsici* KACC 40978, *Fusarium oxysporum* KACC 41083, *Fusarium solani* KACC 41092, *Phytophthora capsici* KACC 40157, *Rhizoctonia solani* KACC 40111 and *Sclerotinia sclerotiorum* KACC 41065 which were obtained from the Korean Agricultural Culture Collection (KACC), Suwon, Republic of Korea. Cultures of fungal species were maintained on potato-dextrose-agar (PDA), containing 4 g per liter potato infusion solids and 20 g per liter dextrose (Acumedia Manufacturers, Inc. Lansing, Michigan, USA) slants and were stored at 4°C.

Preparation of Spore Suspension and Test Samples

The spore suspensions of test pathogens obtained from 10 day old cultures were prepared in sterile distilled water. A haemocytometer was used to obtain a homogenous spore suspension of 1×10^8 spores mL^{-1}. To prepare the stock solutions of essential oil and leaf extracts, the oil was dissolved in dimethylsulfoxide (DMSO) separately, whereas the leaf extracts were dissolved in their respective solvents (hexane, chloroform, ethyl acetate and methanol). Samples with known weights were further diluted with 5% of the respective solvents used

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to prepare test samples, where the final concentration of the solvent was 0.5% (v/v).

Antifungal Activity Assay

Petri dishes (9 cm diameter) containing 20 ml of PDA were used for antifungal activity assay, performed on solid media by the disc diffusion method (Duru *et al*., 2003). Sterile Whatman paper discs of 6 mm diameter were placed on the agar, equidistant and near the border using vernier caliper, where the essential oil (750 µg disc^{-1}) and the leaf extracts (1,500 µg disc^{-1}) were added separately. An agar plug of fungal inoculums (6 mm diameter) was removed from a previous culture of all the fungal strains tested, and placed in the center of the Petri dishes. The plates were incubated at 25ºC for 5 to 7 days, until the growth in the control plates reached the edge of the plates. The plates without the essential oil extracts were used as the negative control. The plates were prepared in triplicate for each treatment. The relative growth inhibition of treatments compared to negative control was calculated by percentage, using the following formula:

Inhibition (%)= [1-Radial growth of treatment (mm)/Radial growth of control (mm)]×100

Determination of Minimum Inhibitory (MIC) and Minimum Fungicidal (MFC) Concentrations

The minimum inhibitory concentrations (MICs) of the essential oil and leaf extracts were determined by two-fold dilution method against *B. cinerea* KACC 40573, *C. capsici* KACC 40978*, F. oxysporum* KACC 41083*, F. solani* KACC 41092*, P. capsici* KACC 40157 and *S. sclerotiorum* KACC 41065 (Gulluce *et al*., 2004). Four and eight micro-liters of oil and extract samples were dissolved in 5% dimethylsulfoxide (DMSO) and the solvents (hexane, chloroform, ethyl acetate and methanol) used for the extraction, respectively. These solutions were serially diluted with their respective 5% solvent and were added to potato dextrose broth (PDB) medium to final concentrations of 32.25, 62.5, 125, 250, 500, 1,000, 2,000, 4,000 and 8,000 µg mL^{-1}. A 10 µL spore suspension (10^{8} spores mL^{-1}) of each test strain was inoculated in the test tubes in PDB medium and incubated for 2-7 days at 28ºC. The control tubes containing PDB medium were inoculated only with fungal spore suspension. The standard reference drug, oligochitosan, was used as the positive control for the tested plant pathogens, which was obtained from Sigma Chemicals (St. Louis, MO). The minimum concentrations at which no visible growth was observed were defined as MICs. Further, the concentrations showing complete inhibition of visual growth of fungal pathogens were identified, and 50 µl of each diluted culture broth was transferred onto the agar plates. The plates were incubated for the specified time and at temperature as mentioned above. The complete absence of growth on the agar surface at the lowest concentration of sample was defined as MFC.

Spore Germination Assay

For spore germination assay of *B. cinerea* KACC 40573, *C. capsici* KACC 40978*, F. oxysporum* KACC 41083*, F. solani* KACC 41092*, P. capsici* KACC 40157 and *S. sclerotiorum* KACC 41065, essential oil samples (2 µL) were dissolved in 5% DMSO to obtain 31.25, 62.5, 125, 250, 500 and 1,000 µg mL^{-1} concentrations of the oil, where the final concentration of DMSO was 0.5% (Leelasuphakul *et al*., 2008). The samples were inoculated with the spore suspension of each fungal pathogen containing 1×10^{8} spores mL^{-1}. From this, aliquots of 10 µL spore suspension from each were placed on separate glass slides in triplicate. Slides containing the spores were incubated in a moisture chamber at 25ºC for 24 hours. Each slide was then fixed in lactophenol-cotton blue and observed under the microscope for spore germination. The spores that generated germ tubes were enumerated and percentage of spore germination was calculated. The control (0.5%

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DMSO) was tested separately for spore germination of different fungi.

Growth Kinetics Assay

P. capsici KACC 40157 which appeared to be a more resistant fungus compared to *F. solani* KACC 41092 to the essential oil in the spore germination assay was chosen as the test fungus for kinetic study and evaluation of antifungal activity of essential oil. A 10 µL spore suspension (about 1×10^8 spores mL^{-1}) of this fungal species was inoculated to different concentrations of essential oil (31.25, 62.5 and 125 µg mL^{-1}) in a test tube and a homogenous suspension was made by inverting the test tubes 3-4 times. After specific intervals of 30, 60, 90, 120 and 150 minutes, the reaction mixtures were filtered through Whatman No. 1 filter paper and the retained spores were washed two or three times with sterile distilled water. The filter was then removed and spores were washed off into 10 ml of sterile distilled water. From this, a 100 µl fraction of spore suspension was taken onto glass slides and incubated at 24±2ºC for 24 hours. The spores that generated germ tubes were enumerated and percentage of spore germination was calculated. Control sets were prepared in 0.5% DMSO with sterile distilled water. All experiments were conducted in triplicate.

Antifungal Activity *In Vivo*

Based on the *in vitro* susceptibility, *P. capsici* KACC 40157 (leaf spot/scorch) was selected as the test fungus for the *in vivo* study conducted on greenhouse grown pepper plants. The *in vivo* antifungal activity of test samples was determined by a whole plant method (Lee *et al*., 2001) and the methodology was adopted as described previously (Bajpai *et al*., 2009).

To prepare the test solutions at the concentration of 1,000 µg ml^{-1}, 4 µL of essential oil was dissolved in 5% dimethylsulfoxide (DMSO) followed by dilution with water containing a surfactant Tween-20 (200 µg mL^{-1}), where the final concentrations of dimethylsulfoxide and Tween-20 were 0.5 and 0.1%, respectively. The initial concentration of the test solution was 1,000 µg mL^{-1}, in further; test dilutions of 500 and 250 µg mL^{-1} of essential oil were employed. For applying the test samples of the oil, four mL of each test sample solution was sprayed onto each pot at the same time. Further, six milliliters of fungal spore suspension (1×10^8 spores mL^{-1}) of *P. capsici* KACC 40157 was sprayed onto each pot. Controls were sprayed with dimethylsulfoxide (0.5%) and Tween-20 (0.1%) solutions. Oligochitosan was used as a reference positive control. The area of lesions on treated plants was measured in millimeters using a Vernier caliper. All tests were conducted in three replicates.

The antifungal efficacy of the test samples on the disease was evaluated after 12 days as a percentage of inhibition calculated by the formula:

Percent inhibition (%)= [(A-B)/A]×100, where *A* and *B* represent the disease area on the untreated and treated plants, respectively.

Statistical Analysis

Analysis of variance for individual parameters was performed by Duncan's multiple range test on the basis of mean values to find out the significance at $P<$ 0.05.

RESULTS

Antifungal Activity

As shown in Table 1, the oil (750 µg $disc^{-1}$) showed potent inhibitory effects on the growth of *B. cinerea* KACC 40573 (59.6%), *C. capsici* KACC 40978 (61.3%), *F. oxysporum* KACC 41083 (58.0%), *F. solani* KACC 41092 (65.6%), *P. capsici* KACC 40157 (59.0%) and *R. solani* KACC 40111 (55.3%). *F. solani* KACC 41092 was found

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Table 1. Antifungal activity of essential oil (750 µg $disc^{-1}$) of *Magnolia liliflora* against plant pathogenic fungi.

Fungal pathogen	Antifungal activity		EO[a]		OC[b]
	mm[c]	%[d]	MIC[e]	MFC[f]	MIC
Fusarium oxysporum KACC 41083	19.0±1.0	58.0±2.0c	500	1000	>2000
Fusarium solani KACC 41092	15.6±0.5	65.6±1.1a	125	125	>2000
Phytophthora capsici KACC 40157	18.6±1.5	59.0±3.6bc	125	250	580
Colletotrichum capsici KACC 41078	17.6±1.1	61.3±2.8b	125	250	>2000
Sclerotinia sclerotiorum KACC 41065	28.0±1.7	38.0±3.4d	500	1000	1500
Botrytis cinerea KACC 40573	18.3±1.5	59.6±3.5bc	500	500	1640
Rhizoctonia solani KACC 40111	20.3±0.5	55.3±1.1c	na	na[g]	na

[a] Essential oil; [b] Oligochitosan (positive control, values in µg ml^{-1}); [c] Radial growth of fungus in PDA plate (in millimeter); [d] Percentage of radial growth inhibition in PDA plate by disc diffusion assay; [e] Minimum inhibitory concentration (values in µg ml^{-1}); [f] Minimum fungicidal concentration (values in µg ml^{-1}), [g] Not applicable. Values are given as Mean ± SD (n= 3) and considered to be significantly different at $P< 0.05$.

to be the most inhibited fungal pathogen by the essential oil. On the other hand, the methanol extract (1,500 µg $disc^{-1}$) exhibited strong antifungal effect (30.0~57.3%) against the tested plant pathogens (Table 2). Chloroform and ethyl acetate extracts also exerted strong antifungal effect with growth inhibition percentages ranging from 33 to 53.3% and 34.6 to 54.6%, respectively. However, chloroform and ethyl acetate extracts displayed low inhibitory effects against *S. sclerotiorum* KACC 41065 (Table 2). Also, hexane extract did not reveal significant results of antifungal activity against the tested plant pathogens (Table 2).

Minimum Inhibitory (MIC) and Minimum Fungicidal (MFC) Concentrations

According to the results given in the Table 1, the minimum inhibitory (MIC) and minimum fungicidal (MFC) concentrations of the essential oil that resulted in complete growth inhibition of *F. oxysporum* KACC 41083, *F. solani* KACC 41092, *P. capsici* KACC 40157, *C. capsici* KACC 40978, *S. sclerotiorum* KACC 41065 and *B. cinerea* KACC 40573 were found in the range of 125 to 500 and 125 to 1,000 µg mL^{-1}, respectively. *F. solani* KACC 41092, *P. capsici* KACC 40157 and *C. capsici* KACC 40978 were found to be the most susceptible fungal pathogens to the essential oil (Table 1). In this study, in most of the cases, the oil exhibited a higher antifungal effect than that of standard oligochitosan with respect to the plant pathogenic fungi tested (Table 1). On the other hand, the MIC and MFC values of methanol, ethyl acetate and chloroform leaf extracts of *M. liliflora* were found to be in the range of 500 to 4,000 and 500 to 8,000 µg mL^{-1}, respectively (Table 3). However, hexane extract did not show any antifungal effect as MIC or MFC values against any of the plant pathogens tested.

Spore Germination

The results for the essential oil from the spore germination assay are shown in Figure 1. DMSO (0.5%, v/v) as a negative control did not inhibit the spore germination of any of the plant pathogens tested. A complete inhibition of fungal spore germination was observed for *F. solani* KACC 41092 and *P. capsici* KACC 40157 at 62.5 and 125 µg mL^{-1} concentrations of essential oil, respectively. Essential oil also exhibited a potent inhibitory effect on the spore germination of *F. oxysporum* KACC 41083, *S. sclerotiorum* KACC 41065, *B. cinerea* KACC 40573 and *C. capsici* KACC 40978 in the range of 20 to 80% at concentrations ranging from 125 to 1,000 µg mL^{-1}.

Table 2. Antifungal activity of various leaf extracts (1500 µg disc^{-1}) derived from *Magnolia liliflora*.

Fungal pathogen	Antifungal activity							
	MLext[a]		HLext[b]		CLext[c]		ELext[d]	
	mm[e]	%[f]	mm	%	mm	%	mm	%
F. oxysporum KACC 41083	20.3±0.5	55.3±1.1a	40.3±1.5	11.0±3.6a	25.6±1.1	43.3±2.8b	21.6±1.1	52.3±2.8ab
F. solani KACC 41092	19.3±0.5	57.3±1.1a	nd[g]	nd	21.3±0.5	53.3±1.1a	20.6±1.1	54.6±2.3a
P. capsici KACC 40157	21.6±1.1	52.3±2.8b	nd	nd	26.0±1.0	42.6±2.5b	24.3±0.5	46.3±1.1b
C. capsici KACC 41078	24.3±1.5	46.3±3.0bc	nd	nd	28.3±1.1	37.3±2.3c	23.3±0.5	48.3±1.1b
S. sclerotiorum KACC 41065	31.6±1.5	30.0±3.6d	nd	nd	41.0±1.0	9.3±2.5d	41.6±1.1	7.6±2.3d
B. cinerea KACC 40573	28.3±1.6	37.3±3.0d	nd	nd	30.3±1.5	33.0±3.6c	29.3±1.5	35.3±3.0c
R. solani KACC 40111	26.3±1.5	42.0±3.6c	41.6±1.5	8.0±3.6b	30.0±1.0	34.0±2.0c	29.6±1.1	34.6±2.3c

[a] Methanol leaf extract; [b] Hexane leaf extract; [c] Chloroform leaf extract; [d] Ethyl acetate leaf extract. [e] Radial growth of fungus in PDA plate (in millimeter); [f] Percentage of radial growth inhibition in PDA plate by disc diffusion assay, [g] Antifungal activity not detected. Values are given as Mean ± SD (n= 3) and considered to be significantly different at $P< 0.05$.

Table 3. Determination of minimum inhibitory (MIC) and minimum fungicidal (MFC) concentrations of various leaf extracts derived from *Magnolia liliflora*.

Fungal pathogen	MIC[a]				MFC[b]			
	MLext[c]	HLext[d]	CLext[e]	ELext[f]	MLext	HLext	CLext	ELext
F. oxysporum KACC 41083	500	nd[g]	500	500	500	nd	500	500
F. solani KACC 41092	500	nd	500	500	500	nd	1000	1000
P. capsici KACC 40157	500	nd	1000	500	1000	nd	2000	500
C. capsici KACC 41078	1000	nd	1000	1000	2000	nd	2000	2000
S. sclerotiorum KACC 41065	2000	nd	nd	nd	4000	nd	nd	nd
B. cinerea KACC 40573	2000	nd	4000	2000	4000	nd	8000	2000

[a] Minimum inhibitory concentration (values in µg mL^{-1}); [b] Minimum fungicidal concentration (values in µg mL^{-1}); [c] Methanol leaf extract; [d] Hexane leaf extract; [e] Chloroform leaf extract; [f] Ethyl acetate leaf extract, [g] Antifungal activity not detected.

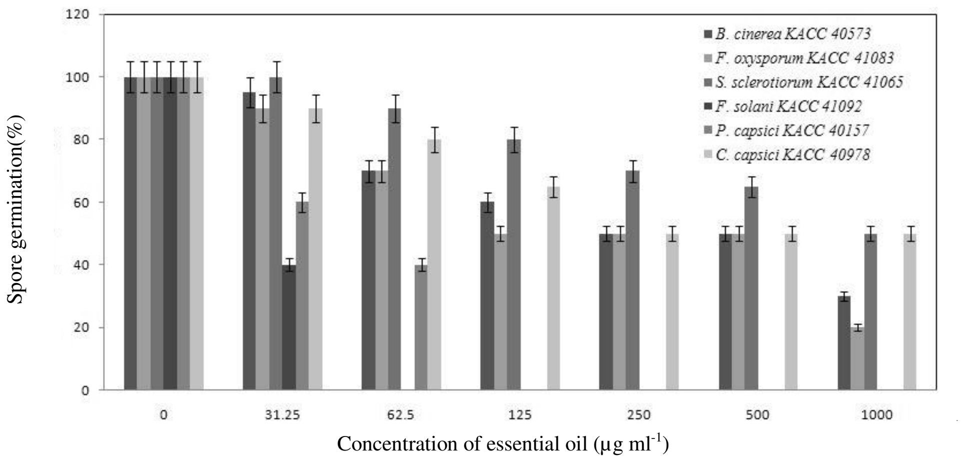

Figure 1. Effect of different concentrations (µg mL^{-1}) of the essential oil of *Magnolia liliflora* on spore germination of tested plant pathogenic fungi. Values are given as Mean±SD (n= 3) and considered to be significantly different at *P*< 0.05.

Growth Kinetics

The antifungal kinetics of the essential oil against *P. capsici* KACC 40157 is shown in Figure 2. Exposure of *P. capsici* spores to different concentrations of the essential oil for a period of 0 to 150 min caused varying degrees of inhibition of spore germination. An increase in fungicidal activity was observed with increase in exposure time and concentration. The essential oil at 31.25 µg mL^{-1} showed antifungal activity but not rapid killing and about 35% inhibition was observed at the exposure time of 120 minutes. However, there was a marked increase in the killing rate at 62.5 and 125 µg mL^{-1} after 30 minutes of exposure, and 80% and 100% inhibitions of spore germination were observed at 150 minutes exposure, respectively.

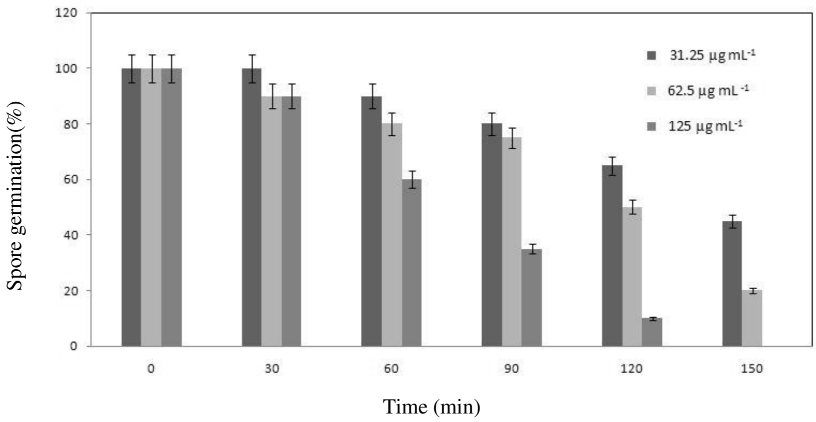

Figure 2. Kinetics of inhibition of *Phytophthora capsici* KACC 40157 spores by the essential oil of *Magnolia liliflora*. Values are given as Mean±SD (n= 3) and considered to be significantly different at *P*< 0.05.

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In Vivo Antifungal Activity

According to the results given in Table 4 and Figure 3, the oil exhibited a wide range of *in vivo* antifungal activity. At the initial concentration of 1,000 μg mL^{-1} the oil exhibited 100% antifungal effect against leaf spot/scorch of pepper caused by *P. capsici* KACC 40157. Further dilutions of the oil applied onto the plants were 500 and 250 μg mL^{-1}. Also at the concentration of 500 μg mL^{-1}, the potential antifungal effect of the oil was observed with 100% antifungal effect against *P. capsici* KACC 40157. However, the oil at the concentration of 250 μg mL^{-1} had a moderate antifungal effect (83.23%) against *P. capsici* KACC 40157 (Table 4). It was observed that the antifungal

Table 4. *In vivo* antifungal activity of essential oil of *Magnolia liliflora* against plant pathogenic fungus of *P. capsici* KACC 40157 on greenhouse grown pepper plants.

Group	Treatment	Applied concentration (μg mL^{-1})	Disease suppression efficacy (%)
Control (Normal)	-	0	- [a]
Control (Vehicle only)	VH [b]	0	-
Control + Pathogen	VH	0	0.0 ± 0.0
PC [c]	-	5%	58.0 ± 1.2
Treatment [e]	VH+EO [d]	250	83.23 ± 0.63
	VH+EO	500	100.0 ± 0.0
	VH+EO	1,000	100.0 ± 0.0

[a] Normal control plant without treatment or vehicle only having no disease symptoms; [b] Vehicle solution (0.5% DMSO+0.1% Tween-20 in water); [c] Positive control: Oligochitosan; [d] Essential oil, [e] Treatment in vehicle solution.

Figure 3. *In vivo* antifungal activity of the essential oil of *M. liliflora* against plant pathogenic fungus of *Phytophthora capsici* KACC 40157 on greenhouse grown pepper plants. a: Treated with pathogen (*P. capsici* KACC 40157) in vehicle; b: No treatment (normal control); c: Treated with vehicle (0.5% DMSO+0.1% Tween-20 in water); d, e, f: Treated with pathogen and different concentrations of essential oil (1,000, 500 and 250 μg mL^{-1}, respectively) in vehicle.

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effect of leaf essential oil was rapid and it exhibited a remarkable antifungal effect as compared to the standard antifungal agent oligochitosan (Table 4).

DISCUSSION

The increasing social and economic implications caused by fungi mean there is a constant striving to produce safer food crops and to develop new antifungal agents. In general, essential oils are considered as non-phytotoxic compounds and potentially effective in food and agriculture industries against pathogenic fungi (Pandey *et al*., 1982; Bajpai *et al*., 2007). In recent years, interests have been generated in the development of safer antifungal agents such as plant based essential oils and extracts to control phytopathogens in agriculture (Costa *et al*., 2005; Bajpai and Kang, 2010).

In brief, the hydrodistillated oil of *M. liliflora* contained oxygenated mono- and sesquiterpenes, and their respective hydrocarbons (Bajpai *et al*., 2008). In recent years, several researchers have reported the mono- and sesquiterpenes, and mono- and sesquiterpene hydrocarbons as the major components of various essential oils from plant origin, which have enormous potential to strongly inhibit the growth of microbial pathogens (Gudzic *et al*., 2002; Cakir *et al*., 2004).

In the present study, the essential oil of *M. liliflora* showed potential *in vitro* and *in vivo* antifungal effects against the tested plant pathogens. Earlier *in vitro* and *in vivo* studies on the analysis of antifungal effect of various oil/extracts showed that they had varying degrees of antifungal effect against different plant pathogenic fungi (Al-Reza *et al*., 2010; Bajpai *et al*., 2007; Yoo *et al*., 1998). As shown in this study, the oil and leaf extracts of *M. liliflora* exhibited strong antifungal effects as MIC and MBC values as well as inhibitory effects against the tested plant pathogens in spore germination and growth kinetic assays. The essential oil of *M. liliflora* also showed a potential *in vivo* antifungal effect against the tested plant pathogen on greenhouse grown pepper plants, inhibiting the growth of *P. capsici* KACC 40157 with 100% disease suppression efficacy at the applied oil concentrations of 500 and 1,000 μg mL^{-1}, and these results were in strong agreement with our previous findings (Al-Reza *et al*., 2010). These activities could be attributed to the presence of α-terpineol, α-bourbonene, *β*-caryophyllene, 2-*β*-pinene, *α*-humulene, farnesene and caryophyllene oxide components of *M. liliflora* oil as evident by the previous findings of Chang *et al*. (2008). Research on the analysis and antifungal properties of the essential oils of various species have shown that they had varying degrees of growth inhibitory effects against plant pathogenic fungi due to their different chemical compositions (Alvarez-Castellanos *et al*., 2001; Singh *et al*., 2002; Bouchra *et al*., 2003).

Certain plant extracts and phytochemicals act in many ways on various types of disease complex, and may be applied in food and agro- industries in the same way as other chemical fungicides. *Magnolia liliflora* mediated oil and extracts can also be used as a leading factor in a wide range of activities against many plant pathogenic fungi, where these pathogens have developed resistance against specific fungicides (Elad, 1991). Besides, minor components present in our essential oil such as farnenol, linalool oxide, geraniol, isobornyl acetate, myrcenyl acetate, geranyl acetate, and α-muurolene may also contribute to the antifungal activity of the oil involving some type of synergism with the other active components (Marino *et al*., 2004).

The development of natural antimicrobials and fungal pesticides would help to decrease the negative impact of synthetic agents, such as residues, resistance and environmental pollution. In this respect, natural fungicides may be effective, selective, biodegradable, and less toxic to the environment as well as food and agriculture industries. Thus, it can be concluded that the use of *M. liliflora*

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mediated oil and extracts could be considered as an antifungal available to develop novel types of natural fungicides and to control several plant pathogenic fungi causing severe fungal diseases in food, crops and vegetables.

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اثر بازدارندگی روغن ضروری و عصاره های ***Magnolia liliflora* Desr.** در محیط‌های *in vitro* و *in vivo* در برابر قارچ‌های بیماری‌زای گیاهی

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چکیده

این مطالعه به منظور بررسی کارایی بازدارندگی روغن ضروری و عصاره‌های به‌دست آمده از گل و برگ *Magnolia liliflora* Desr. در محیط‌های *in vitro* و *in vivo* در برابر قارچ‌های بیماری‌زای گیاهی به انجام رسید. روغن (۷۵۰ $\mu g\ disc^{-1}$) و عصاره‌های مختلف برگ از جمله هگزان، کلروفرم، اتیل استات، و متانول (۱۵۰۰ $\mu g\ disc^{-1}$) اثرات ضدقارچی رضایت بخشی در برابر *Botrytis cinerea*

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Fusarium oxysporum KACC ،*Colletotrichum capsici* KACC 40978 ،KACC 40573 41083، *Fusarium solani* KACC 41092، *Phytophthora capsici* KACC 40157، *Rhizoctonia solani* KACC 40111و*Sclerotinia sclerotiorum* KACC 41065 با درصدهای بازدارندگی رشد شعاعی به ترتیب برابر ۳۸-۶۵/۶٪ و ۷/۶-۵۷/۳٪ و مقادیر MIC و MFC بین ۱۲۵-۵۰۰ و ۱۲۵-۱۰۰ μg mL^{-1}و ۵۰۰-۴۰۰۰ و ۵۰۰-۸۰۰۰ μg mL^{-1}به دست دادند. روغن اثر مخرب شدیدی بر جوانه زنی هاگ‌ها در همه گیاهان مورد آزمون و نیز غلظت و سینتیک بازدارندگی وابسته به زمان *P. capsici* KACC 40157 داشت. همچنین، این روغن اثر ضد قارچ *in vivo* شدیدی در برابر *P. capsici* KACC 40157یکی از عوامل بیماری‌زای انتخاب شده در گیاهان فلفل گلخانه‌ای از خود نشان داد. نتایج این مطالعه نشان دادند که می توان از روغن گل و عصاره‌های برگ *M. liliflora* به‌عنوان یک جایگزین طبیعی برای قارچ کش‌های مصنوعی به منظور کنترل رشد برخی از قارچ‌های بیماری‌زای مهم گیاهی در شرایط *in vitro* و*invivo* استفاده نمود.

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