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.

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
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, 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 1×10⁸ 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%
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:

\[
\text{Percent inhibition} (\%) = \frac{(A-B)}{A} \times 100, \\
\]

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

<table>
<thead>
<tr>
<th>Fungal pathogen</th>
<th>Antifungal activity</th>
<th>EO(^a)</th>
<th>OC(^b)</th>
<th>MIC(^c)</th>
<th>MFC(^d)</th>
<th>MIC(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fusarium oxysporum</em> KACC 41083</td>
<td>19.0±1.0</td>
<td>58.0±2.0</td>
<td>500</td>
<td>1000</td>
<td>&gt;2000</td>
<td></td>
</tr>
<tr>
<td><em>Fusarium solani</em> KACC 41092</td>
<td>15.6±0.5</td>
<td>65.6±1.1</td>
<td>125</td>
<td>125</td>
<td>&gt;2000</td>
<td></td>
</tr>
<tr>
<td><em>Phytophthora capsici</em> KACC 40157</td>
<td>18.6±1.5</td>
<td>59.0±3.6</td>
<td>bc</td>
<td>125</td>
<td>250</td>
<td>580</td>
</tr>
<tr>
<td><em>Colletotrichum capsici</em> KACC 40178</td>
<td>17.6±1.1</td>
<td>61.3±2.8</td>
<td>125</td>
<td>250</td>
<td>&gt;2000</td>
<td></td>
</tr>
<tr>
<td><em>Sclerotinia sclerotiorum</em> KACC 41065</td>
<td>28.0±1.7</td>
<td>38.0±3.4</td>
<td>500</td>
<td>1000</td>
<td>1500</td>
<td></td>
</tr>
<tr>
<td><em>Botrytis cinerea</em> KACC 40573</td>
<td>18.3±1.5</td>
<td>59.6±3.5</td>
<td>bc</td>
<td>125</td>
<td>500</td>
<td>1640</td>
</tr>
<tr>
<td><em>Rhizoctonia solani</em> KACC 40111</td>
<td>20.3±0.5</td>
<td>55.3±1.1</td>
<td>na</td>
<td>na(^g)</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

\(a\) 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.

<table>
<thead>
<tr>
<th>Fungal pathogen</th>
<th>MExt&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HExt&lt;sup&gt;b&lt;/sup&gt;</th>
<th>CLext&lt;sup&gt;c&lt;/sup&gt;</th>
<th>EExt&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. oxysporum KACC 41083</td>
<td>20.3±0.5</td>
<td>55.3±1.1a</td>
<td>40.3±1.5</td>
<td>11.0±3.6a</td>
</tr>
<tr>
<td>F. solani KACC 41092</td>
<td>19.3±0.5</td>
<td>57.3±1.1a</td>
<td>nd&lt;sup&gt;b&lt;/sup&gt;</td>
<td>nd</td>
</tr>
<tr>
<td>P. capsici KACC 40157</td>
<td>21.6±1.1</td>
<td>52.3±2.8b</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>C. capsici KACC 40178</td>
<td>24.3±1.5</td>
<td>46.3±3.0bc</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>S. sclerotiorum KACC 41065</td>
<td>21.6±1.5</td>
<td>30.0±3.6d</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>B. cinerea KACC 40573</td>
<td>28.3±1.6</td>
<td>37.3±3.0d</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>R. solani KACC 40111</td>
<td>26.3±1.5</td>
<td>42.0±3.6c</td>
<td>41.6±1.5</td>
<td>8.0±3.6c</td>
</tr>
</tbody>
</table>

<sup>a</sup>Methanol leaf extract; <sup>b</sup>Hexane leaf extract; <sup>c</sup>Chloroform leaf extract; <sup>d</sup>Ethyl acetate leaf extract. Radial growth of fungus in PDA plate (in millimeter); Percentage of radial growth inhibition in PDA plate by disc diffusion assay. 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.

<table>
<thead>
<tr>
<th>Fungal pathogen</th>
<th>MIC&lt;sup&gt;c&lt;/sup&gt;</th>
<th>MFC&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. oxysporum KACC 41083</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>F. solani KACC 41092</td>
<td>500</td>
<td>nd</td>
</tr>
<tr>
<td>P. capsici KACC 40157</td>
<td>500</td>
<td>nd</td>
</tr>
<tr>
<td>C. capsici KACC 40178</td>
<td>1000</td>
<td>nd</td>
</tr>
<tr>
<td>S. sclerotiorum KACC 41065</td>
<td>2000</td>
<td>nd</td>
</tr>
<tr>
<td>B. cinerea KACC 40573</td>
<td>2000</td>
<td>nd</td>
</tr>
</tbody>
</table>

<sup>a</sup>Minimum inhibitory concentration (values in μg mL⁻¹); <sup>b</sup>Minimum fungicidal concentration (values in μg mL⁻¹); <sup>c</sup>Methanol leaf extract; <sup>d</sup>Hexane leaf extract; Chloroform leaf extract; Ethyl acetate leaf extract, 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.
**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.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Applied concentration (µg mL⁻¹)</th>
<th>Disease suppression efficacy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal)</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Control (Vehicle only)</td>
<td>VH</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Control + Pathogen PC</td>
<td>VH</td>
<td>0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Treatment e</td>
<td>VH+EO</td>
<td>250</td>
<td>83.23 ± 0.63</td>
</tr>
<tr>
<td></td>
<td>VH+EO</td>
<td>500</td>
<td>100.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>VH+EO</td>
<td>1,000</td>
<td>100.0 ± 0.0</td>
</tr>
</tbody>
</table>

* 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.
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*
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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**Plant Pathogenic Fungi and Magnolia Liliflora**

در *Magnolia liliflora* Desr. انر بازدارنگی روغن ضروری و عصاره های محیط‌های *in vivo* و *in vitro* در برای قارچ‌های بیماری‌زا گیاهی و ک. باچیای، و س. ج. کاتگ

چکیده

این مطالعه به منظور بررسی بazăدارنگی روغن ضروری و عصاره‌های بهدست آمده از گل و در محیط‌های *Magnolia liliflora* Desr. برگ می‌باشد. روغن (150 µg disc⁻¹) و عصاره‌های مختلف برگ از چمله هگران، کارفورم، *Botrytis cinerea* اثرات ضدفارقی رضایت بخشی در برای
Fusarium oxysporum KACC 40978, Colletotrichum capsici KACC 40573, Rhizoctonia solani KACC 40157, Phytophthora capsici KACC 40111, Sclerotinia sclerotiorum KACC 40165.

M. liliflora M. liliflora

P. capsici KACC 40157

M. liliflora M. liliflora

P. capsici KACC 40157

M. liliflora M. liliflora

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