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Screening for inhibitory activities of essential oils on the growth of Colletotrichum gloeosporioides (Penz.) Penz. & Sacc., the causal agent of leaf spot disease of Murraya koenigii L.

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Screening for inhibitory activities of essential oils on the growth of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., the causal agent of leaf spot disease of *Murraya koenigii* L.

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Seven essential oils namely clove, cedar wood, lemongrass, peppermint, eucalyptus, citronella and neem oils were tested for their inhibitory effect on spore germination, growth of germ tube and mycelial growth of *Colletotrichum gloeosporioides* isolated from diseased *Murraya koenigii*. All essential oils inhibited the germination and growth of germ tube at different concentrations. However, significant reduction in colony growth was observed with citrus, lemongrass and peppermint oils at 1000, 1500 and 2000 ppm concentrations, respectively. Citrus oil at 1360 ppm inhibited the maximum growth of the fungus followed by lemongrass oil at 1720 ppm and peppermint at 2260 ppm, respectively. The effect of essential oils on mycelial dry weight also showed antifungal activity on the growth of *Colletotrichum gloeosporioides*. The study revealed the possible utilisation of these essential oils for foliar spray for the management of leaf spot disease of *Murraya koenigii*.

**Keywords:** *Murraya koenigii*; leaf spot disease; essential oils, inhibitory effect; *Colletotrichum gloeosporioides*

**Introduction**

*Murraya koenigii* L. (Rutaceae) commonly known as curry vegetable is also a medicinal plant grown throughout the greater part of India and south-east Asia and is used as an antibacterial, anti-inflammatory and antifeedant agent in ayurvedic medicinal preparations (Kirthikar and Basu 1935). The leaf spot disease of *Murraya koenigii* caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (teleomorph *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk) reduces its utility in food industry (Smith and Black 1990). Chemical management of the disease is not suitable as this method leaves behind the chemical residues (Daferera et al. 2003). Antimicrobial activities of the essential oil obtained from some medicinal plants revealed that some of them exhibited antibacterial, antifungal and insecticidal properties (Burt 2004). Numerous studies have documented the antifungal properties of essential oils against various fungal species (Bouchra et al. 2003; Sokmen et al. 2004). They are complex volatile compounds produced in different plant parts, which
are known to have various function in plants including conferring pest and disease resistance (Goubran and Holmes 1993). The natural plant products that are readily available, biodegradable, non-phytotoxic and environmentally friendly are very useful in plant disease management. Hence, in the present investigation, essential oils were evaluated against the fungi *Colletotrichum gloeosporioides* for their antifungal activity for the management of leaf spot disease in *Murraya* in vivo.

**Materials and methods**

**Sources of essential oils**

Essential oils such as clove, cedar wood, lemongrass, peppermint, eucalyptus, citronella and neem oils were obtained from the Karnataka Aromas Essential Oil Distillery, Bangalore, Karnataka. Eucalyptus oil was purchased from the Venus Eucalyptus Oil Distillery, Nilgiris, Tamilnadu, India.

**Fungal isolates**

*Colletotrichum gloeosporioides* was isolated from leaf spot-affected *Murraya koenigii* collected from different regions of Mysore District (Karnataka State). The fungal isolates were maintained on potato dextrose agar (PDA) medium for further use.

**Effect of essential oils on spore germination, germ tube growth and colony diameter of Colletotrichum gloeosporioides**

The spore suspension of *Colletotrichum gloeosporioides* was prepared in 0.1% yeast extract and 0.1% sucrose with phosphate buffer, and the suspension was adjusted to 1 × 10^6 spores/ml by using Haemocytometer. Five different concentrations of all the seven essential oils (500 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm) were prepared and evaluated for their effect on spore germination and the length of germ tube by incubation at 25°C for 72 h. After incubation, per cent spore germination and germ tube growth were measured using micrometry. The spores were considered to have germinated when the germ tubes were equal in length or more than the spore size.

The inhibitory effects of essential oils were tested on *Colletotrichum gloeosporioides* by agar dilution method (Fraternale et al. 2003). Briefly, PDA medium (20 ml) was mixed with requisite amount of each oil separately to prepare 500, 1000, 1500, 2000 and 2500 ppm concentrations; 0.005% (v/v) Tween 80 was used as an emulsifying agent. Agar disk (5 mm) was taken from five-day-old culture of *Colletotrichum gloeosporioides* placed on the centre of each agar plate and incubated at 25 ± 2°C for 16 days. Three replicates were maintained for each treatment. The inhibitory effect was evaluated by measuring fungal colony diameter by using a centimetre scale. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined and compared with the control.

**Effect of essential oils on mycelial wet and dry weight**

The effect of essential oils on the mycelial wet and dry weight was determined by liquid culture method (Mukhopadhyay and Nandi 1997). Potato dextrose broth was used as basal medium for the growth of *Colletotrichum gloeosporioides*. Briefly,
100 ml of potato dextrose broth in 250 ml Erlenmeyer flasks, containing different concentrations, 0, 500, 1000, 1500, 2000 and 2500 ppm, of essential oils were inoculated with 5-mm mycelial discs of *Colletotrichum gloeosporioides* and incubated at 25 ± 2°C for 16 days. The mycelial mats were filtered through dried pre-weighed Whatman 45 filter disc and the wet weight was noted. The mycelial mats were then dried at 110°C in a hot air oven for 16 h and the dry weight was determined.

**Statistical analysis**

Statistical methods were applied to calculate means and standard deviations for all the data. Statistical analysis ANOVA was used to know the effect of essential oils on mycelial growth, spore germination, growth of germ tube and wet and dry weights, respectively.

**Results**

*Effect of essential oils on spore germination and germ tube growth*

The effect of essential oils on the spore germination and growth of germ tube is presented in Tables 1 and 2. The spore germination was significantly inhibited by all essential oils with increasing concentrations of oils tested when compared to control after 24 h of incubation. However, among seven essential oils tested, citrus, lemongrass and peppermint oils significantly inhibited the spore germination and spore growth with increasing concentrations of essential oils.

*Effect of essential oils on fungal colony diameter*

Inhibitory effect of essential oils on the fungal colony diameter is presented in Table 3. All essential oils showed inhibitory effect on the growth of *Colletotrichum gloeosporioides* with increasing concentrations. Among the seven essential oils tested, lemongrass, peppermint and citrus oils showed high inhibitory effect when compared to eucalyptus, cedar wood and neem oils. At 2500 ppm of concentrations of lemongrass, peppermint and citrus oils, there was no growth of *Colletotrichum gloeosporioides*. The MIC of citrus, lemongrass and peppermint oil was found to be 1000, 1500 and 2000 ppm while the MFC was found to be 1360, 1720 and 2260 ppm, respectively. The significant decrease in wet and dry weight of the test fungus was also observed with increased in the concentrations of essential oils tested (Figures 1 and 2).

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Control</th>
<th>Peppermint</th>
<th>Cedar wood</th>
<th>Lemongrass</th>
<th>Citrus</th>
<th>Eucalyptus</th>
<th>Neem</th>
<th>F value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>176.33</td>
<td>90.00</td>
<td>111.33</td>
<td>26.00</td>
<td>28.33</td>
<td>94.00</td>
<td>76.67</td>
<td>90.26</td>
<td>0.00</td>
</tr>
<tr>
<td>1000</td>
<td>188.67</td>
<td>68.67</td>
<td>69.00</td>
<td>12.33</td>
<td>18.33</td>
<td>73.33</td>
<td>67.33</td>
<td>1360</td>
<td>0.00</td>
</tr>
<tr>
<td>1500</td>
<td>189.67</td>
<td>41.67</td>
<td>54.33</td>
<td>10.00</td>
<td>00.00</td>
<td>33.33</td>
<td>61.67</td>
<td>1720</td>
<td>0.00</td>
</tr>
<tr>
<td>2000</td>
<td>195.33</td>
<td>0.00</td>
<td>33.00</td>
<td>2.670</td>
<td>00.00</td>
<td>32.33</td>
<td>45.33</td>
<td>2260</td>
<td>0.00</td>
</tr>
<tr>
<td>2500</td>
<td>176.33</td>
<td>0.00</td>
<td>13.67</td>
<td>0.00</td>
<td>00.00</td>
<td>27.67</td>
<td>22.33</td>
<td>1089.46</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*Values given are mean of 10 readings.*
Table 2. Effect of different concentrations of essential oils on the growth of germ tube after 24 h of incubation.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Essential oils (growth of conidial germ tube in range, μm*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lemongrass</td>
</tr>
<tr>
<td>Control</td>
<td>115–180</td>
</tr>
<tr>
<td>500</td>
<td>28–30</td>
</tr>
<tr>
<td>1000</td>
<td>21–30</td>
</tr>
<tr>
<td>1500</td>
<td>17–25</td>
</tr>
<tr>
<td>2000</td>
<td>00–00</td>
</tr>
<tr>
<td>2500</td>
<td>00–00</td>
</tr>
</tbody>
</table>

*Values given are mean of 10 readings.

Table 3. Effect of essential oils on mycelial growth of *Colletotrichum gloeosporioides*.

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Concentrations (ppm)</th>
<th>Essential oils (fungus colony diameter, cm*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Peppermint</td>
</tr>
<tr>
<td>1</td>
<td>500</td>
<td>9.00</td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>9.00</td>
</tr>
<tr>
<td>3</td>
<td>1500</td>
<td>9.00</td>
</tr>
<tr>
<td>4</td>
<td>2000</td>
<td>9.00</td>
</tr>
<tr>
<td>5</td>
<td>2500</td>
<td>9.00</td>
</tr>
</tbody>
</table>

*Values given are mean of three readings.

Figure 1. Effect of different concentrations of essential oils on the dry and wet weight of *Colletotrichum gloeosporioides*. 
The antimicrobial properties of essential oils and their constituents have been the subject of many investigations during the past decades (Basilico and Basilico 1999). The main reason for suitability of essential oil is their natural origin, which consumers find comforting and which are less hazardous for the environment, and the very low risk that pathogens would develop resistance to the mixture of components that make up the oils with their apparent diversity of antifungal mechanisms (Martos et al. 2007). Many investigators have documented the antimicrobial activity of various essential oils against different microbial species (Chao and Young 2000). In this study, the ability of seven essential oils to inhibit the fungal pathogen of leaf spot disease-causing agent of *Murraya koenigii* was investigated. The data revealed that all the tested essential oils showed broad spectrum antifungal activity against *Colletotrichum gloeosporioides*. Furthermore,
citrus, lemongrass and peppermint oils were very effective in inhibiting the spore germination and growth of germ tube and in reducing the wet and dry weight of Colletotrichum gloeosporioides. The spore germination and germ tube formation are prerequisite for successful infection (Nielsen et al. 2000). Some studies have shown that specific essential oils can control the growth rate and spore germination of fungi (Hope et al. 2003). The inhibition of germ tube by essential oils and the effectiveness of these essential oils in reducing the germination and germ tube length appear to be important in preventing pathogenesis. It is reported that secondary metabolites of the plants confer resistance against fungal infection by disrupting the membrane as well as by preventing cell wall synthesis (Ghosh et al. 2005). The inhibition could be due to a broad spectrum antifungal activity of various essential oils against Colletotrichum gloeosporioides. The other reason could be that the essential oils cause a reduction in hyphal diameter that may cause alterations in fungal metabolism (Zani et al. 1991). The extent to which these essential oils contribute to the antifungal activity on Colletotrichum gloeosporioides warrants further study. The present study revealed the application of essential oils as protectants against leaf spot disease of Murraya koenigii in vivo after further screening.

References

