**Short Communication**

*In vitro* antifungal study of the efficacy of some plant extracts for inhibition of *Alternaria carthami* fungus

Abhijit Ranaware*, Vrijendra Singh and Nandini Nimbkar
Nimbkar Agricultural Research Institute, P.O. Box 44, Tambmal, Phaltan-415 523, Maharashtra, India

Received 29 June 2009; Accepted 27 April 2010

The antifungal activity of aqueous extracts of seven plant species, viz. bulbs of Garlic (*Allium sativum* Linn.) and leaves of Datura (*Datura metel* Linn.), Lantana (*Lantana camara* Linn.), Eucalyptus (*Eucalyptus* hybrid), Neem (*Azadirachta indica* A. Juss.), Madagascar periwinkle (*Catharanthus roseus* G. Don) and Holy basil (*Ocimum sanctum* Linn.) against safflower leaf spot disease-causing fungus, *Alternaria carthami*, under *in vitro* conditions was investigated. Linear growth reduction of the pathogen on PDA plates at 20% plant extract concentration was recorded seven days after inoculation. *A. sativum* bulb extract exhibited maximum inhibition of the pathogenic fungus (48.68%) followed by *D. metel* (44.25%), while *O. sanctum* extract showed the least inhibitory effect (25.02%). The observations indicate the efficacy of aqueous plant extracts as potential inhibitors of *Alternaria carthami*.

**Keywords:** *Alternaria carthami*, Antifungal, *Allium sativum*, *Ocimum sanctum*, *Datura metel*.

**IPC code; Int. cl.**—A61 K 36/00

**Introduction**

Safflower (*Carthamus tinctorius* Linn.) is an important *Rabi* oilseed crop in India. It has superior adaptability to scanty moisture conditions and it also yields oil, rich in polyunsaturated fatty acids (linoleic acid 78%) which help in reducing the blood cholesterol level.

Safflower is susceptible to some major fungal diseases such as *Alternaria* leaf spot, wilt and rust. *Alternaria* leaf spot disease of safflower caused by *Alternaria carthami* is endemic to India and causes huge yield losses. Control of this plant disease by chemicals is costly and also causes environmental pollution which is hazardous to human and animal health. Therefore, alternate means and ways should be found to control the plant diseases. Several higher plants and their constituents have been successfully used in plant disease control. Use of antifungal plant extracts as a component of integrated disease management can prove useful. The present study was carried out to compare the efficacy of different plant extracts for inhibition of *Alternaria carthami* under *in vitro* conditions.

**Materials and Methods**

**Pathogen**

Naturally infected leaves showing *Alternaria* leaf spot symptoms were collected from field-cultivated safflower plants at the Nimbkar Agricultural Research Institute, Phaltan. The leaf tissue exhibiting characteristic dark brown spots with concentric rings was removed for isolating the pathogen. These leaf tissue samples were treated with 0.1% HgCl₂ solution, rinsed with sterilized distilled water and pat-dried on sterile blotting paper. The affected leaf tissue was incised and placed on PDA media plates. After seven days of incubation at 30°C, the fungal growth was removed for isolating the pathogen. These leaf tissue samples were treated with 0.1% HgCl₂ solution, rinsed with sterilized distilled water and pat-dried on sterile blotting paper. The affected leaf tissue was incised and placed on PDA media plates. After seven days of incubation at 30°C, the fungal growth was removed for isolating the pathogen. These leaf tissue samples were treated with 0.1% HgCl₂ solution, rinsed with sterilized distilled water and pat-dried on sterile blotting paper. The affected leaf tissue was incised and placed on PDA media plates. After seven days of incubation at 30°C, the fungal growth was removed for isolating the pathogen. These leaf tissue samples were treated with 0.1% HgCl₂ solution, rinsed with sterilized distilled water and pat-dried on sterile blotting paper. The affected leaf tissue was incised and placed on PDA media plates. After seven days of incubation at 30°C, the fungal growth was removed for isolating the pathogen. These leaf tissue samples were treated with 0.1% HgCl₂ solution, rinsed with sterilized distilled water and pat-dried on sterile blotting paper. The affected leaf tissue was incised and placed on PDA media plates. After seven days of incubation at 30°C, the fungal growth was removed for isolating the pathogen. These leaf tissue samples were treated with 0.1% HgCl₂ solution, rinsed with sterilized distilled water and pat-dried on sterile blotting paper. The affected leaf tissue was incised and placed on PDA media plates. After seven days of incubation at 30°C, the fungal growth was removed for isolating the pathogen. These leaf tissue samples were treated with 0.1% HgCl₂ solution, rinsed with sterilized distilled water and pat-dried on sterile blotting paper. The affected leaf tissue was incised and placed on PDA media plates. After seven days of incubation at 30°C, the fungal growth was removed for isolating the pathogen.
Plant material for extracts

Fresh samples of seven plant species, viz. bulbs of Garlic (Allium sativum Linn.) and leaves of Datura (Datura metel Linn.), Lantana (Lantana camara Linn.), Eucalyptus (Eucalyptus hybrid), Neem (Azadirachta indica A. Juss.), Madagascar periwinkle (Catharanthus roseus G. Don) and Holy basil (Ocimum sanctum Linn.) were used for the experiment.

Preparation of aqueous plant extracts

Two grams of fresh leaves or bulbs were cleaned with tap water, then surface-sterilized with 0.1% HgCl₂ for 1 minute followed by rinsing with sterilized distilled water (SDW) to ensure removal of traces of HgCl₂ if any. The leaves or bulbs were finely chopped with a sterile blade and then macerated using 2 ml of SDW in a sterilized porcelain mortar with the help of a sterilized pestle. The plant extracts obtained were filtered through a thin layer of sterilized cotton. The final volume was made up to 10 ml by adding SDW and stored in refrigerator at 4ºC until use. This concentration (20%), as a crude aqueous extract was used in the antifungal activity experiment.

Linear mycelial growth assay

One ml of aqueous plant extract (20%) was poured into sterilized petri dishes (9 cm diam.) followed by 15 ml of sterilized PDA medium and gently swirled to facilitate uniform mixing of plant extract and PDA medium. After the PDA medium solidified, center of each petri dish was inoculated with a 5 mm disc of seven days’ old culture of A. carthami. Three plates were used for each extract as replicates. Control plates contained PDA medium alone. The plates were incubated at 28±2ºC for seven days. The linear growth of pathogenic fungus was measured in centimeters and the linear growth reduction percentage was calculated using the formula given below.

\[
\text{Linear growth reduction (\%) = } \left( \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \right) \times 100
\]

Results and Discussion

The results of effect of plant extracts on the linear mycelial growth of pathogenic fungus A. carthami under in vitro condition are presented in Table 1.

It shows that the linear mycelial growth of A. carthami was inhibited on PDA medium containing plant extracts when compared to the control. Seven days after inoculation, there was free growth in control (3.9 cm) (Plate 1). The plates consisting of PDA and the bulb extract of Garlic caused significant reduction in the radial growth of pathogenic fungus (1.7 cm) and maximum inhibition (48.68 %) (Plate 2). It was at par with the leaf extract of Datura metel Linn. which limited the radial growth to 2.0 cm giving significant inhibition of pathogen (44.25%) (Plate 3). These two extracts were significantly superior to the remaining ones. However, the remaining five extracts gave significantly higher linear growth reduction percentages compared to the untreated control.

The fungitoxic effect of A. sativum bulb extract against Alternaria solani⁵ and A. alternata⁶ has been recorded in potato crop. Also the A. sativum bulb extract has been reported to significantly reduce the radial growth and spore germination of A. tenuis⁷ and Colletotrichum capsici⁸ in chilli. The leaf extracts of D. metel and Catharanthus roseus G. Don were reported to inhibit the conidial germination of Phaeoisariopsis personata in groundnut⁹ and Bipolaris sorokiniana in wheat¹⁰. Efficacy of leaf extracts of L. camara against A. solani has also been

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Plant extract</th>
<th>Mean linear mycelial growth (cm)</th>
<th>Linear growth reduction percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Allium sativum</td>
<td>1.7</td>
<td>56.4 (48.68)*</td>
</tr>
<tr>
<td>2.</td>
<td>Datura metel</td>
<td>2.0</td>
<td>48.7 (44.25)</td>
</tr>
<tr>
<td>3.</td>
<td>Lantana camara</td>
<td>2.8</td>
<td>28.2 (32.08)</td>
</tr>
<tr>
<td>4.</td>
<td>Eucalyptus hybrid</td>
<td>2.9</td>
<td>25.6 (30.40)</td>
</tr>
<tr>
<td>5.</td>
<td>Azadirachta indica</td>
<td>3.0</td>
<td>23.1 (28.73)</td>
</tr>
<tr>
<td>6.</td>
<td>Catharanthus roseus</td>
<td>3.1</td>
<td>20.5 (26.92)</td>
</tr>
<tr>
<td>7.</td>
<td>Ocimum sanctum</td>
<td>3.2</td>
<td>17.9 (25.02)</td>
</tr>
<tr>
<td>8.</td>
<td>Control</td>
<td>3.9</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*Figures in parentheses indicate arc-sin values.

Plate 1— Growth of Alternaria carthami on PDA medium (Control plates, 7 days old culture)
shown in potato. Inhibition of sclerotia production in sheath blight of paddy and of *A. solani* in potato, using aqueous leaf extract of *Eucalyptus globulus* has been reported. A significant linear growth reduction *in vitro* of *A. alternata* in sunflower due to leaf extracts of *A. indica* has also been reported. *O. sanctum* and *A. sativum* extracts have been reported to inhibit spore germination of *A. brassicaceae* in mustard crop.

**Conclusion**

The present study has shown the potential of aqueous plant extracts as effective inhibitors of *Alternaria carthami*. However, further investigations are required to test the efficacy of plant extracts in managing *Alternaria carthami* under field conditions and their effect if any on the growth of the crop.

**References**