Efficacy of volatile metabolites of phylloplane fungi of *Rauwolfia serpentina* against *Alternaria alternata*

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Abstract

Diseases of medicinal plants cause great economic losses and the use of chemical fungicides poses threat not only to the environment but also to the health of human beings. Now-a-days due to the hazardous effect of chemical fungicides application of biocontrol agents is being adopted. In the present study, phylloplane fungi of *Rauwolfia serpentina* were screened as potential biocontrol agents to assess their antagonistic potential against *Alternaria alternata*. Ten fungi were isolated from phylloplane of *R. serpentina*. Volatile metabolites produced from *Trichoderma harzianum* ISO-1 showed maximum reduction in mycelial growth of *A. alternata* followed by *T. harzianum* ISO-2, *T. piluliferum*, *Aspergillus niger* and *P. sublateritium*.

Key words – Biocontrol agents – medicinal plant – pathogen

Introduction

*Rauwolfia serpentina* (L.). Benth. ex Kurz. belongs to the family Apocynaceae and is commonly known as *sarpagandha*. *R. serpentina* is an important medicinal plant of Indian subcontinent and South East Asian countries. Reserpine is an alkaloid first isolated from *R. serpentina* and was widely used as an antihypertensive drug (Fabricant & Fransworth 2001, Harisaranraj et al. 2009, Dey & De 2010). The plant is used in traditional medicinal practices for treatment of various central nervous system disorders associated with psychosis, schizophrenia, insanity, insomnia, epilepsy and acts as hypnotics (Pakrashi & Akkhari 1968, Meena et al. 2009).

*Alternaria alternata* Keissler is one of the important plant pathogens reported as causing leaf spot disease of *Rauwolfia serpentina* (Puni & Harsh 2009). Chemical control of the pathogen is known but the intensive use of fungicides has resulted in the accumulation of toxic compounds potentially hazardous to humans and the environment and also in the built-up of resistance in the pathogens.

An environment friendly approach is the introduction of biological control agents. Biological control is the inhibition of growth, infection or reproduction of one organism using another organism (Baker 1987, Cook 1993). The phylloplane (leaf surface) provides a suitable habitat for the growth of antagonistic microorganisms which can compete with the pathogens for nutrients and inhibit pathogen multiplication by secreting antibiotics or toxins. Biological approaches for the control of pathogens on aerial surfaces have been reviewed by (Blakeman &
Fokkema 1982, Andrew 1990, 1992, Elad 1993). Adebanjo & Bankole (2004), Euveh et al. (2011) had explored the ability of certain antagonistic fungi for the possible control of pathogenic fungi on aerial plant surfaces. The present study was carried out to examine the efficacy of volatile metabolites produced by phylloplane fungi of *Rauwolfia serpentina* against *Alternaria alternata* under *in vitro* conditions.

**Materials and Methods**

**Isolation of leaf pathogen**

Leaves of *R. serpentina* infected with *A. alternata* were collected from Dr. Sushila Tiwari Medicinal Plant Nursery, Rishikesh, Uttarakhand. For the isolation of pure culture of fungal pathogen, a portion of leaf containing circular brown spot was surface sterilized by submerging in 0.1% mercuric chloride for 1 min, after which it was rinsed with three changes of sterilized distilled water. Then, they were placed on potato dextrose agar medium in Petri plates and incubated in a B.O.D. incubator at 25±1°C for mycelial growth.

**Isolation of phylloplane fungi**

Phylloplane fungi were isolated from healthy leaves of *R. serpentina* through leaf washing technique (Dickinson 1967, Aneja 2003) and identified with standard monographs and expertise available. To study their antagonistic properties pure cultures were maintained on potato dextrose agar medium at 4°C in a refrigerator for further studies.

**Effect of volatile metabolites from antagonists on the growth of *A.alternata***

The method described by Dennis & Webster (1971) was followed to study *in vitro* effect of volatile metabolites of the leaf surface fungi on the test pathogen. Petri dishes of 7 cm diameter containing 10 ml PDA medium were inoculated with a 5 mm agar block of each phylloplane fungus in triplicate. The Petri dishes were incubated at 25±1°C for a week. The lid of each Petri dish was replaced by the bottom of another Petri dish containing 10 ml PDA medium with 5 mm agar block of the *A. alternata* and sealed together with parafilm and re-incubated at 25±1°C. For control, the lids of uninoculated Petri dishes containing PDA medium were sealed in the same way with bases of Petri dishes containing the test pathogen. Radial growth of *A. alternata* was measured after 48, 72 and 96 h. The growth inhibition (%) of the pathogen was calculated by the following formula:

\[
\text{Per cent growth inhibition} = \frac{(C-T)}{C} \times 100
\]

Where, C = Growth in control

\[ T = \text{Growth in treatment} \]

**Results**

Ten phylloplane fungi were identified as *viz. Trichoderma harzianum* Rifai ISO-1 and ISO-2, *T. piluliferum* Webster and Rifai, *Aspergillus niger* van Tieghem, *Penicillium sublateritium* Biourge, *P. herquei* Bainier and Sartory, *P. frequentans* Westling, *P. tardum* Thom, *P. citreo-viride* Biourge and *Cladosporium cladosporioides* (Fresen.) de Vries. While the pathogen was identified as *A. alternata*. Growth inhibition of *A. alternata* in presence of antagonists is shown in (Fig.1).

Results showed that out of the ten antagonists *T. harzianum* ISO-1 (44.60%) showed the maximum inhibition of mycelial growth of *A. alternata* followed by *T. piluliferum* (36.24%) which was at par with *T. harzianum* ISO-2 (35.50%) (Table1). Metabolites produced by *A. niger* (22.73%) was at par with *P. sublateritium* (23.56%) and showed moderate level of inhibition. *P. tardum* showed minimum (7.47%) mycelial growth inhibition. Whereas, *P. herquei* (14.76%), *C. cladosporioides* (13.70%), *P. citreo-viride* (9.71%) and *P. frequentans* (9.09%) were found to be at par with each other and comparatively less effective in inhibiting the mycelial growth of the pathogen.
Fig.1 – Effect of volatile metabolites produced from different phylloplane fungi on the growth of *Alternaria alternata* in Petri plates a Control. b *A. niger*, c *C. cladosporioides*. d *P. frequentans*. e *P. citreo-viride*. f *P. herquei*. g *P. sublateritium*. h *P. tardum*. i *T. harzianum* ISO-1. j *T. harzianum* ISO-2. k) *T. piluliferum*.

**Table1** Evaluation of volatile metabolites produced by phylloplane fungi against the test pathogen *A. alternata*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Antagonist</th>
<th>Per cent inhibition of mycelial growth (Mean±S.D.)</th>
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<tbody>
<tr>
<td>1.</td>
<td><em>A. niger</em></td>
<td>22.73±3.18</td>
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<tr>
<td>2.</td>
<td><em>C. cladosporioides</em></td>
<td>13.70±1.27</td>
</tr>
<tr>
<td>3.</td>
<td><em>P. citreo-viride</em></td>
<td>9.71±1.92</td>
</tr>
<tr>
<td>4.</td>
<td><em>P. frequentans</em></td>
<td>9.09±2.26</td>
</tr>
<tr>
<td>5.</td>
<td><em>P. herquei</em></td>
<td>14.76±1.43</td>
</tr>
<tr>
<td>6.</td>
<td><em>P. sublateritium</em></td>
<td>23.56±3.19</td>
</tr>
<tr>
<td>7.</td>
<td><em>P. tardum</em></td>
<td>7.47±6.81</td>
</tr>
<tr>
<td>8.</td>
<td><em>T. harzianum</em> ISO-1</td>
<td>44.60±2.24</td>
</tr>
<tr>
<td>9.</td>
<td><em>T. harzianum</em> ISO-2</td>
<td>35.50±1.32</td>
</tr>
<tr>
<td>10.</td>
<td><em>T. piluliferum</em></td>
<td>36.24±2.84</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>21.74</td>
</tr>
<tr>
<td>SEM ±</td>
<td></td>
<td>1.77</td>
</tr>
<tr>
<td>CD at 5%</td>
<td></td>
<td>5.22</td>
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</table>
Discussion
Disease management of medicinal plants needs to be focused on the utilization of potential biocontrol agents instead of chemicals. The production of volatile and non-volatile antibiotics by the species of *Trichoderma* was reported by (Dennis & Webster 1971, Ubalua & Oti 2007). Volatile compounds released from *Trichoderma* species were able to arrest and inhibit the hyphal growth of various plant pathogenic fungi (Doi & Mori 1994).
Pandey & Upadhyay (1997) reported the effectiveness of diffusible volatile compounds produced by *T. harzianum* in vitro. Ghildyal & Pandey (2008) also estimated that *Trichoderma* sp. produced diffusible and volatile metabolites. Faheem *et al.* (2010) reported that *T. harzianum* inhibited the mycelial growth of *Alternaria brassicicola*. Gveroska & Ziberoski (2011) examined that volatile metabolites produced by *T. harzianum* efficiently reduced the mycelial growth of *A. alternata*. *Penicillium* spp. were also found capable of producing volatile antibiotics in agar (Jayasuriya *et al.*. 1996). The findings of present study are in conformity with the above mentioned studies.

Conclusion
*In vitro* findings demonstrated that *Trichoderma* spp., *A. niger* and *P. sublateritium* were found to exhibit higher antagonistic efficiency in inhibiting the mycelial growth of *A. alternata* through the production of volatile metabolites. Therefore, application of phylloplane fungi as biocontrol agents can be suggested as an effective and ecofriendly approach in comparison to the synthetic fungicides for the management of leaf spot disease in *Rauwolfia serpentina*.

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References
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