Current Research in Environmental & Applied Mycology 4 (2): 152–156 (2014) ISSN 2229-2225

www.creamjournal.org **Article** CREAM

Online Edition

Doi 10.5943/cream/4/2/2

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

Thakur S[1*] and Harsh NSK[2]

[1,2] Forest Pathology Division, Forest Research Institute, Dehradun - 248 006 Uttarakhand, India. patho.shikha@gmail.com

Thakur S, Harsh NSK 2014 – Efficacy of volatile metabolites of phylloplane fungi of *Rauwolfia serpentina* against *Alternaria alternata*. Current Research in Environmental & Applied Mycology 4(2), 152–156, Doi 10.5943/cream/4/2/2

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 &

Submitted 30 May 2014, Accepted 2 August 2014, Published online 16 September 2014
Corresponding Author: Shikha Thakur – e-mail – patho.shikha@gmail.com

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±1ºC 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±1ºC 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±1ºC. 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:

Per cent growth inhibition = (C-T) C×100
Where, C = Growth in control
T = 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. piluliferm* (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.

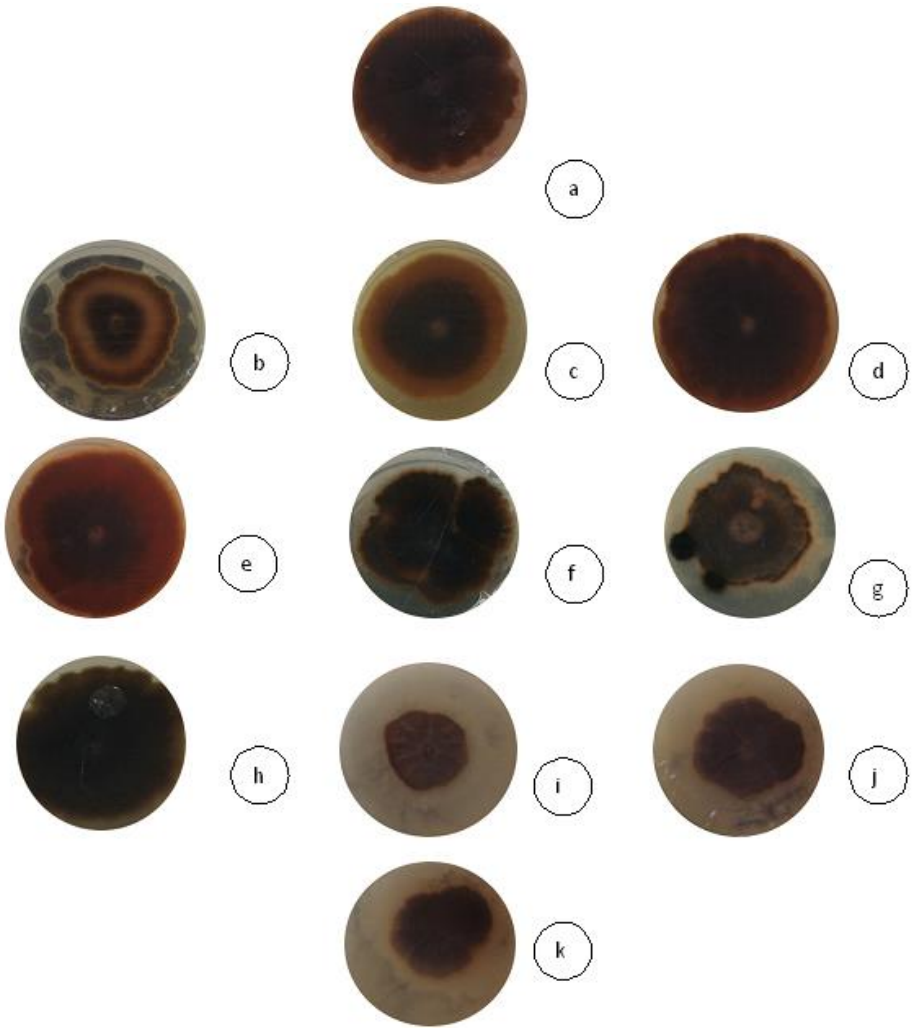

Fig.1 – Effect of volatile metabolites produced from different phylloplane fungi on the growth of *Alternaria alternata* in Petri plates a Control. b *A. niger*, c *C. cladosporioides*. d *P. frequentans*. e *P. citreo-viride*. f *P. herquei*. g *P. sublateritium*. h *P. tardum*. i *T. harzianum* ISO-1. j *T. harzianum* ISO-2, k) *T. piluliferum*.

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

S.No.	Antagonist	Per cent inhibition of mycelial growth (Mean±S.D.)
1.	*A. niger*	22.73±3.18
2.	*C.cladosporioides*	13.70±1.27
3.	*P.citreoviride*	9.71±1.92
4.	*P. frequentans*	9.09±2.26
5.	*P. herquei*	14.76±1.43
6.	*P.sublateritium*	23.56±3.19
7.	*P. tardum*	7.47±6.81
8.	*T. harzianum* ISO-1	44.60±2.24
9.	*T. harzianum* ISO-2	35.50±1.32
10.	*T. piluliferum*	36.24±2.84
Mean		21.74
SEM ±		1.77
CD at 5%		5.22

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.*

Acknowledgement

The authors express their gratitude to the Director, Forest Research Institute, Dehradun, for providing the facilities to complete this study successfully.

References

Adebanjo A, Bankole SA. 2004 – Evaluation of some fungi and bacteria for biocontrol of anthracnose disease of cowpea. Journal of Basic Microbiology 44, 3-9.

Andrew JH 1990 – Biological control in the phyllosphere: Realistic goal or false hope. Canadian Journal of Plant Pathology12, 300–307.

Andrew JH 1992 – Biological control in the phyllosphere. Annual Review of Phytopathology 30, 603–635.

Aneja KR 2003 – Experiments in microbiology, plant pathology and biotechnology, 4th Edn., New age international publications limited.176p.

Baker KF 1987 – Evolving concepts of biological control of plant pathogens. Annual Review of Phytopathology 25, 67–85.

Blakeman JP, Fokkema NJ. 1982 – Potential for biocontrol of plant diseases on the phylloplane. Annual Review of Phytopathology 20, 167–192.

Cook RJ 1993 – Making greater use of introduced microorganisms for biological control of plant pathogens. Annual Review of Phytopathology 31, 53–80.

Dennis C, Webster J. 1971 – Antagonistic properties of species-groups of *Trichoderma* hyphal interaction. Transactions of British Mycological Society 57, 363–369.

Dey A, De JN. 2010 – *Rauwolfia serpentina* (L). Benth. ex.Kurz.- A review. Asian Journal of Plant Sciences 9, 285–298.

Dickinson CH 1967 – Fungal colonization of Pisum leaves. Canadian Journal of Botany. 45, 915–927.

Doi S, Mori M. 1994 – Antifungal properties of metabolites produced by *Trichoderma* isolates from saw dust media of edible fungi against wood decay fungi. Material and Organismen 28,143–151.

Elad Y 1993 - Microbial suppression of infection by foliar plant pathogens. IOBC Bulletin 16,

3 – 7.
Euveh AG, Okhuoya JA, Osemwegie OO, Hamad AI, Ogbebor ON. 2011 – Evaluation of phylloplane fungi as biocontrol agent of Corynespora diseases of rubber (*Hevea brasiliensis* Muell.Arg.).World Journal of Fungal and Plant Biology. 2, 1–5.
Fabricant DS, Farnsworth NR. 2001 – The value of plants used in traditional medicine for drug discovery. Environment Health Perspect 109, 69–75.
Faheem A, Razdan VK, Mohiddin FA, Bhat KA, Sheikh PA. 2010 – Effect of volatile metabolites of *Trichoderma* species against seven fungal plant pathogens *in-vitro*. Journal of Phytology 2, 34–37.
Ghildyal A, Pandey A. 2008 – Isolation of cold tolerant antifungal strains of *Trichoderma* sp. from glacial sites of Indian Nimalayan Region. Research Journal of Microbiology 3, 559–564.
Gveroska B, Ziberoski J. 2011 – *Trichoderma harzianum* as a biocontrol agent against *Alternaria alternata* on tobacco. Applied Technologies and Innovations 7, 67–76.
Harisaranraj R, Suresh K, Saravanababu S. 2009 – Evaluation of the chemical composition *Rauwolfia serpentina* and *Ephedra vulgaris*. Advances in biological research 3, 174–178.
Jayasuriya KE, Deacon JE. 1996 – Possible role of 2-Furaldehyde in the biological control of white root disease of rubber. J. Rubber Res. Inst. 77, 15–77.
Meena AK, Bansal P, Kumar S. 2009 – Plant-herbal wealth as a potential source of ayurvedic drugs. Asian Journal of Traditional Medicines. 4, 152–170.
Pakrashi S, Akkhari B. 1968 – *Rauwolfia* alkaloids. Farmatsiia. 17, 64–82.
Pandey KK, Uapadhyay JP. 1997 – Selection of potential biocontrol agents based on production of volatile and nonvolatile antibiotics. Veg. Sci. 24,140–143.
Puni L, Harsh NSK. 2009 – Studies on nursery diseases of important medicinal plants of Uttarakhand Non wood Forest Products Division Forest Research Institute (Indian Council of Forestry Research and Education). Dehradun.78p.
Ubalua A, Oti E. 2007 – Antagonistic properties of *Trichoderma viride* on post-harvest cassava root rot pathogens. African Journal of Biotechnology. 6, 244–245.