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Research Paper

Biocontrol Potential of Phylloplane Fungi Against Alternaria Alternata Causing Leaf Spot in *Rauwolfia Serpentina in Vitro.*

Science

KEYWORDS : Antagonism, Alternaria alternata, Rauwolfia serpentina, Phylloplane fungi

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ABSTRACT

Potential of phylloplane fungi of Sarpgandha (Rauwolfia serpentina) as biocontrol agents were screened for their antagonistic activities against Alternaria alternata in vitro, causing leaf spot in Rauwolfia serpentina. Culture filtrates of antagonists under "with cell" and "cell free" conditions were examined and showed that T.piluliferum (79.50) *was at par with T.harzianum ISO-1*(76.80) *followed by T.harzianum ISO-2* (64.02)*, P.sublateritium* (59.00) *and A.niger* (50.00)*.whereas, C.cladosporioides showed the minimum suppression* (13.10)*.*

Introduction

Biological control of plant disease is the suppression of disease symptoms and disease incidence by the application of a biological agent, usually a microorganism. The term phyllospere to denote the leaf surface of the plants was introduced by Last (1955). Leaf surface commonly known as phylloplane also provides a habitat for the growth of antagonistic microorganisms which can compete with the pathogen for nutrients, inhibit pathogen multiplication by secreting antibiotics or toxins. Biological control of plant pathogens has been considered as a potential control strategy in recent years and search for these biological agents in increasing. *Trichoderma* spp. are the most commonly used fungal biological control agents and have long been known as effective antagonists against plant pathogenic fungi (Chet *et al*.,1981., Papavizas,1985.,Chet,1987).

Rauwolfia serpentina (L). Benth. ex Kurz. commonly known as Sarpagandha (Apocynaceae) is an important **medicinal plant** of Indian subcontinent and South East Asian countries. It is an erect, evergreen perennial undershrub. Leaves are in whorls of three or four rarely opposite, shining, green above and pale beneath (Dhiman, 2005., Dey *et al*., 2010). *R.serpentina* is widely distributed in the sub-himalayan tract from Punjab eastwards to Nepal, Sikkim and Assam; also in some parts of Central India and Western Ghats (Anon., 1969). *Rauwolfia* can be regarded as a typical drug of ayurvedic medicine. Its roots are used as a valuable remedy for high **blood pressure,** insomnia, anxiety, excitement, schizophrenia, insanity, epilepsy, hypochondria and other disorders of the central nervous system. Besides, the juice of the leaves is applied for removal of opacities of the cornea of the eyes (Monachino,1954., Kirtikar and Basu, 1993.,Wilkin and Judson,1953).

Leaf spot on *Rauwolfia serpentina* is one of the most wide spread disease appears usually in July and August. The disease is caused by *Alternaria alternata* (Fr.) Keissler (Puni and Harsh, 2009). The disease appears as minute yellow spots which gradually increase in size, turns dark and the leaves fall off. Several measures have been carried out to control the disease such as physical,chemical and biological. Although a number of synthetic organic and inorganic fungicides have been developed to control plant diseases. The intensive use of fungicides has resulted in the accumulation of toxic compounds potentially hazardous to humans, environment and also in the built-up of resistance in the pathogens.

The increasing awareness of fungicide-related hazards has emphasized the need of adopting biological control methods as an alternative disease control method. Moreover, effective results of disease management through the use of antagonistic bioagents in the laboratory, glass house and field has been achieved during past several years. With this in view the present work was designed to study the antagonistic activities of the phylloplane fungi against the pathogen of *Rauwolfia serpentina in-vitro.*

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. 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 sterile 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 mycelia growth.

Isolation of phylloplane fungi

Phylloplane fungi were isolated from healthy leaves of *R.serpentina* (L). Benth. ex Kurz. through leaf washing technique (Dickinson,1967., Aneja,2003). *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.citreo-viride* Biourge, *Cladosporium cladosporioides* (Fresen.) de Vries. To study their antagonistic properties pure cultures were maintained on potato dextrose agar medium at 4°C for further studies.

Effect of culture filtrates of antagonists on the pathogens:

Flasks prepared with PDB medium were inoculated with antagonist fungi and incubated in BOD incubator at 25±1°C for 15 days. After 15 days of incubation culture filtrate was divided into two parts. One part was filtered with Whatman No.1 filter paper denoted as 'with cell' culture filtrate and the other part was filtered with a bacterial syringe filter (0.45 micron) denoted as 'cell free' culture filtrate.

PDA Petri plates were prepared. Using a flamed cork borer of 5 mm dia, agar plugs were removed at a distance of 1 cm from the periphery of the plate. 5 mm mycelial disc of test pathogen was cut from the actively growing colony margin and placed in the centre of the Petri plates. These wells were then filled with both culture filtrates (20µl/well) of the antagonist fungi as prepared above in separate plates. Whereas, in control set the wells were filled with sterilized distilled (20µl) water and the test pathogen is placed at the centre. These plates were incubated at 25±1°C for 7 days and the radial growth of the pathogen was measured with the help of metric scale (Dhingra and Sinclair, 1985).

Radial growth inhibition was evaluated according to the formula

$$\% \text{ Growth Inhibition} = R_2\text{-}R_1/R_2 * 100$$

Where,

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R_2 = Radial growth of test pathogen in control

R_1 = Radial growth of test pathogen in treatment

Results

Ten phylloplane fungi were identified viz. *Aspergillus niger* van Tieghem, *Cladosporium cladosporioides* (Fresen.) de Vries, *Penicillium citreo-viride* Biourge, *P. frequentans* Westling, *P.herquei* Bainier and Sartory, *P.sublateritium* Biourge, *P.tardum* Thom, *Trichoderma harzianum* Rifai ISO-1 and ISO-2, *T.piluliferum* Webster and Rifai.

Effect of culture filtrates of antagonists on the pathogen

The radial growth of pathogen was suppressed maximum by the antagonist culture filtrate under the condition 'with cell' when it was compared to 'cell free' condition. In respect to antagonists, *T.piluliferum* recorded the highest percent radial growth inhibition (79.52) of *A.alternata* which was at par with *T.harzianum* ISO -1 (76.80) followed by *T.harzianum* ISO-2 (64.02), *P.sublateritium* (58.90) and *A.niger* (50.08). It was also observed that *C.cladosporioides* showed the minimum suppression (13.17) of *A.alternata* (Table 1).

During the interaction between (antagonists and conditions) it was found that all the antagonists viz., *T.harzianum* ISO-2, *A.niger*, *P.sublateritium*, *P.citreo-viride*, *P.frequentans*, *P.herquei*, *P.tardum* and *C.cladosporioides* showed more suppression under 'with cell' in comparison to the 'cell free' condition except *T.harzianum* ISO -1 and *T.piluliferum* which showed similar effects under both the conditions (Fig 1).

Table 2. Per cent inhibition of radial growth of *A.alternata* in Well in agar

S.No.	Antagonists	Conditions		Mean
		With cell (Mean±S.D.)	Cell free (Mean±S.D.)	
1.	*A.niger*	61.73±3.52	38.43±5.19	50.08
2.	*C. cladosporioides*	22.80±4.00	3.53±0.06	13.17
3.	*P. citreo-viride*	23.67±4.73	16.17 ±3.62	19.92
4.	*P. frequentans*	20.93±5.28	13.33±1.12	17.13
5.	*P. herquei*	42.20±1.75	4.67±0.95	23.43
6.	*P.sublateritium*	72.30±3.48	45.77±0.65	58.90
7.	*P.tardum*	51.17±4.11	25.47±3.62	38.32
8.	*T.harzianum* ISO-1	78.33±0.31	75.27±1.24	76.80
9.	*T. harzianum* ISO-2	80.93±3.43	47.10±1.22	64.02
10.	*T.puliliferum*	81.43±2.48	77.60±1.54	79.52
Mean		53.52	34.73	
		A	C	A x C
		1.25	0.56	1.77
SEM±		3.59	1.60	5.08
CD at 5%		61.73±3.52	38.43±5.19	50.08

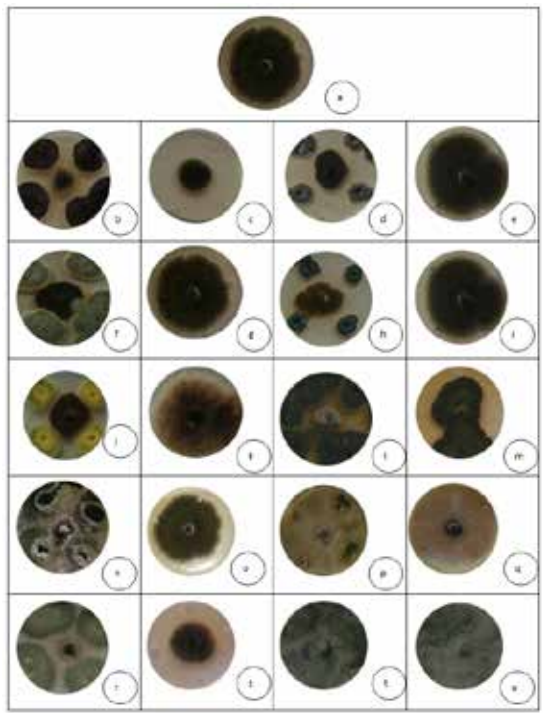


Fig 1. Well in agar method (with cell/cell free) a) Control *Alternaria alternata* b) *A.niger* (with cell) culture filtrate c) *A.niger* (cell free) culture filtrate d) *C.cladosporioides* (with cell) culture filtrate e) *C.cladosporioides* (cell free) culture filtrate f) *P.citreo-viride* (with cell) culture filtrate g) *P.citreo-viride* (cell free) culture filtrate h) *P.frequentans* (with cell) culture filtrate i) *P.frequentans* (cell free) culture filtrate j) *P.herquei* (with cell) culture filtrate k) *P.herquei* (cell free) culture filtrate l) *P.sublateritium* (with cell) culture filtrate m) *P. sublateritium* (cell free) culture filtrate n) *P. tardum* (with cell) culture filtrate o) *P.tardum* (cell free) culture filtrate p) *T. harzianum* ISO-1(with cell) culture filtrate q) *Trichoderma harzianum* ISO-1(cell free) culture filtrate r) *T. harzianum* ISO-2 (with cell) culture filtrate s) *T. harzianum* ISO-2 (cell free) culture filtrate t) *T. piluliferum* (with cell) culture filtrate u) *T.piluliferum* (cell free) culture filtrate.

Discussion

Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Baker and Paulitz, 1996). Culture filtrates of *T. harzianum* ISO-1 and *T. harzianum* ISO-2 effectively inhibited mycelial growth of *A.alternata*. Raju *et al.* (2008) stated that the cultures or cell free filtrates of all the *Trichoderma* spp. viz., *T.harzianum* suppressed the radial growth of *Rhizoctonia solani.* In this study, it was found that 'with cell' culture filtrate were found to be more effective when compared to 'cell free' culture filtrate in inhibiting the radial growth of pathogens. Gveroska and Ziberoski (2012) also observed a strong reducing effect of *T.harzianum* on the development of *Alternaria alternata* which can be further applied in the biological control of this pathogen.

Conclusion

The study has revealed that *Trichoderma harzianum* ISO-1, ISO-2 and *Trichoderma piluliferum* were the most effective in inhibiting the radial colony growth of the test pathogen. while *Aspergillus niger* and *P. sublateritium* were found to be moderately effective. Therefore, the application of phylloplane fungi as biocontrol agents can be recommended as an effective and environment friendly approach in comparison to the synthetic fungicides for the management of leaf spot on *Rauwolfia serpentina.*

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