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Full Length Article

Phylloplane fungi as biocontrol agent against *Alternaria* leaf spot disease of (Akarkara) *Spilanthes oleracea*

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ABSTRACT

In vitro studies were conducted to evaluate the efficacy of phylloplane fungi in controlling the leaf spot disease of *Spilanthes oleracea* caused by *Alternaria alternata*. *Trichoderma harzianum* ISO-1, *T.harzianum* ISO-2 and *T.piluliferum* caused maximum inhibition of test pathogen followed by *Aspergillus niger* and *Penicillium sublateritium* whereas *P.tardum* and *Cladosporium cladosporioides* showed minimum antagonistic efficacy.

Key Words: Antagonisic efficacy, Biocontrol, Phylloplane fungi, *Spilanthes oleracea*

INTRODUCTION

Leaf surface commonly known as phylloplane provides a suitable habitat for the growth of antagonistic microorganisms which can compete with the pathogen for nutrients and inhibit pathogen multiplication by secreting antibiotics or toxins (Blakeman, 1982; Yadav *et al*., 2011). The phyllosphere of plants is a dynamic ecosystem inhabited by specific bacteria, yeasts and fungi. Their activity is related to various interactions between the biotic and abiotic factors of the environment (Behrendt *et al*., 1997, 1999). Interactions of microorganisms living on the surface of the plants aboveground parts are based on antibiosis, competition and parasitism (Chakraborty *et al*., 1994, Stromberg *et al*., 2000) which protect the plants from pathogenic microorganisms and improving their health.

For the management of foliar diseases certain chemical fungicides are known and used. Intensive use of fungicides has not only resulted in the accumulation of toxic compounds, potentially hazardous to humans and the environment (Reshu and Khan, 2012), but also in the build-up of resistance in the pathogens (Heydari and Pessarakli, 2010). As a consequence, they have limited use when plant parts are to be used for herbal preparations consumed by human beings. Biological control for plant diseases is now receiving increasing attention, the potential of biological control through the effect of phyllosphere antagonists has been realized for sometime. Several workers have investigated the use of biological control of plant diseases (Tewari, 1995; Ogbebor and Adekunle, 2005).

Gorawar and Hedge (2006) examined the potential of *Trichoderma* sp. in inhibiting the foliar pathogen *Alternaria alternata.* Similar findings were made by Rajkonda *et al*. (2011) where the inhibitory potential of *Trichoderma* spp. was analysed against five pathogenic fungi *Alternaria alternata*, *Rhizoctonia solani*, *Geotrichum candidum*, *Fusarium oxysporum* and *Macrophomina phaseolina* through dual culture technique and were found to be effective.

***Spilanthes oleracea* Linn. (**Akarkara) **belonging to the family** Asteraceae is a flowering herb also known as 'toothache plant' as the leaves and flower heads contain an analgesic agent spilanthol used to numb toothache. A decoction or infusion of the leaves and flowers is a traditional remedy for stammering, toothache, stomatitis and throat disorders.

It's properties provide relief to dry mouth by enhancing saliva production (Anon., 1976). *S.oleracea* is attacked by *Alternaria alternata* Keissler causing leaf spot disease resulting into failure of crop (Bhandari, 2008; Bhandari *et al*., 2014).

Patale and Mukadam (2010) screened *Trichoderma* species viz., *T. viride*, *T. harzianum*, *Trichoderma* sp. (Local) which were evaluated for their biocontrol potential against plant pathogenic fungi *Aspergillus flavus*, *Phytophthora* sp., *Fusarium oxysporium*, *Rhizoctonia soloni*, *Penicillium notatum* and *Alternaria solani* and found that *Trichoderma* sp. effectively inhibited the growth of pathogenic fungi in the dual culture. Similar findings were made by Singh and Kumar (2011) they examined three isolates of *Trichoderma harzianum* (TH) against *Fusarium oxysporum f. sp. chrysonthemi* (Focl) and found that TH isolates effectively inhibited the growth of pathogenic fungi. Reshu and Khan (2012) analysed the antagonistic efficacy of *Trichoderma harzianum* and *Aspergillus niger* against *Alternaria brassicae* isolated from phylloplane of mustard. Prakasam and Sharma (2012) studied the biocontrol potential of *T.harzianum* in the management of purple blotch of onion caused by *A.porri*.

The present study screens some phylloplane fungi for their antagonistic potential against *Alternaria* leaf spot of *S.oleracea*.

MATERIALS AND METHODS

Isolation of leaf pathogen

Leaves of *S.oleracea* infected with *A.alternata* were collected from Non Wood Forest Products Division Nursery, Forest Research Institute, Dehradun, and Uttarakhand. For the isolation of pure culture of fungal pathogen, a portion of leaf containing brown spot was surface sterilized with 0.1% mercuric chloride for 1 min, followed by rinsing with three changes of sterilized distilled water and was placed on potato dextrose agar medium in Petri plates. The plates were 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 *S.oleracea* through leaf washing technique (Dickinson, 1967; Aneja, 2003) and identified with standard monographs (Ellis, 1971) and expertise available. To study their antagonistic properties pure cultures were maintained on potato dextrose agar medium at 4°C in a refrigerator.

***In vitro* colony interaction** (Dual culture technique)

Sterilized potato dextrose agar medium was poured aseptically into sterilized Petri dishes of 7 cm dia. Dual inoculation of the pathogen and an antagonist was set up. Culture discs of 5 mm dia were cut from the periphery of the actively growing colonies using a sterilized cork borer. Disc of test fungus was placed aseptically at the edge of the Petri plate. These plates were incubated at 25±1°C for 3 days. Mycelial disc (5 mm) of antagonist was inoculated on opposite side of Petriplate three days after the pathogen to adjust for the slow growth rate of the pathogens. Paired cultures were again incubated at 25±1°C for 6-9 days and observed periodically. Then antagonistic fungi were tested against *A.alternata*. Each set was made in 3 replicates.

Antagonistic behaviour was measured quantitatively by calculating the area. Graph paper was used to measure the area of the antagonists, test pathogen species and inhibition zone in the Petri plate. Antagonistic efficacy for each antagonist against the pathogen was worked out according to the following formula (Ojha, 2000):

Antagonistic efficacy = b + c - a

Where,

a = % of area of test pathogen sp. with antagonist in the same Petri plate (cm^2)

b = % of area of antagonist, and

c = % area of inhibition zone between antagonist and pathogen or overgrowth of antagonist over test fungus.

RESULTS AND DISCUSSION

Potential antagonists tried were identified based on their cultural and microscopic characteristics as *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.

Antagonism between colonies of *Alternaria alternata* and phylloplane fungi

Mycelial growth measurement of *A.alternata* and the ten antagonists towards each other on Potato Dextrose Agar on the tenth day

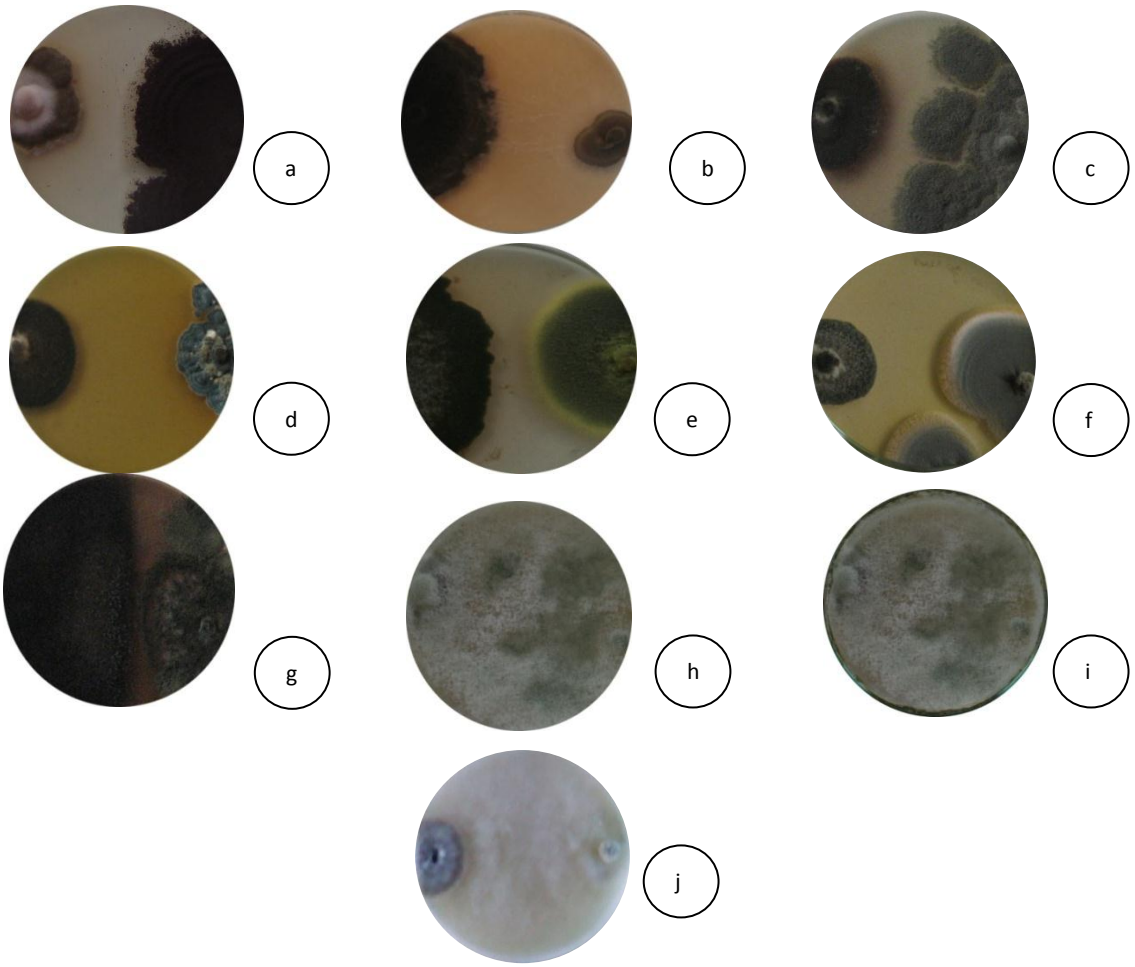

Fig.1. a-j Screening of antagonistic fungi against *A.alternata* a) *A.niger*, b) *C.cladosporioides*, c) *P.citereo-viride*, d) *P.frequentans*, e) *P.herquei*, f) *P.sublateritium*, g) *P.tardum*, h) *T.harzianum* ISO-1, i) *T.harzianum* ISO-2, j) *T.piluliferum*.

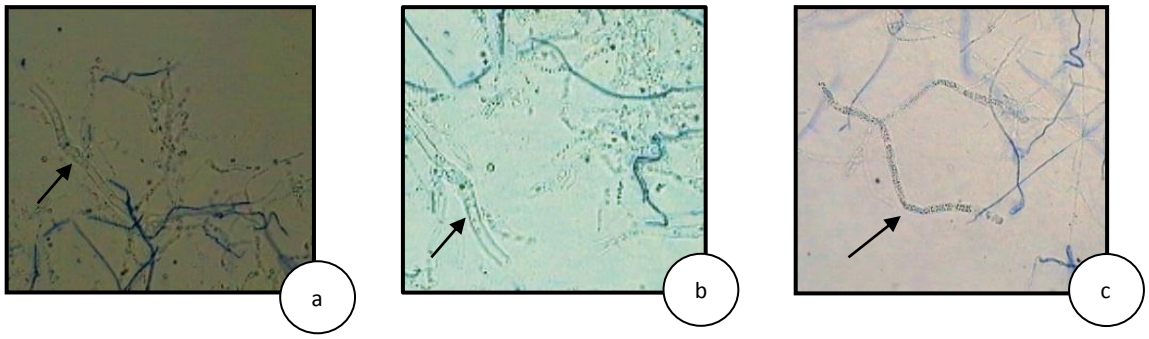

Fig 2. Lysis in the hyphae of *A.alternata* caused by a) *T.harzianum* ISO-1, b) *T.harzianum* ISO-2, c) *T.piluliferum*.

after inoculation and percent inhibition of *A.alternata* are summarized in Table 1. A significant interaction was exhibited by *Trichoderma* isolates in which growth of *A.alternata* was affected as the *antagonistic* fungi grew over the colony of *A.alternata* and completely inhibited its growth. *Trichoderma harzianum* ISO-1, *T.harzianum* ISO-2 and *T.piluliferum* inhibited the growth of *A.alternata* by 90.00% and exhibit maximum efficacy followed by *A.niger* (67.40%) and *P.sublateritium* (64.13%). Antagonists *P. citreo-viride* (63.39%), *P. herquei* (55.28%) and

Table 1: Antagonistic efficacy of antagonist isolates against *A.alternata*

Sr. No.	Antagonists	Percent Antagonistic efficacy (Mean±S.D.)
1	*A. niger*	67.40(85.07)±3.44
2	*C. cladosporioides*	27.57(21.43)±0.79
3	*P. citreo-viride*	63.39(79.93)±0.70
4	*P. frequentans*	37.37(37.07)±7.17
5	*P. herquei*	55.28(67.47)±3.75
6	*P. sublateritium*	64.13(80.93)±1.34
7	*P. tardum*	24.05(16.67)±2.00
8	*T. harzianum* ISO-1	90.00(100)±0.00
9	*T.harzianum* ISO-2	90.00(100)±0.00
10	*T.plililiferum*	90.00(100)±0.00
Mean		60.92
SEM±		1.67
CD at 5%		4.94

*Original values are given in parentheses

P. frequentans (37.37%) were also found to be effective in inhibiting the growth of *A.alternata* whereas *P.tardum* (24.05%) and *C.cladosporioides* (27.57%) showed the minimum antagonistic efficacy against *A.alternata* (Fig.1).

Microscopic observations of mycelial interactions

Microscopic observations were carried out on the interaction of *A.alternata* with *Trichoderma harzianum* ISO-1, *T.harzianum* ISO-2 and *T.piluliferum* in which lysis of the hyphae occurred and the pathogen could not be re-isolated from the point of contact (Fig.2). Antagonists *A.niger*, *P.sublateritium*, *P.citreo-viride*, *P.frequentans*, *P.herquei*, *P.tardum* and *C.cladosporioides* did not show any clear pattern of hyphal interaction.

Trichoderma harzianum is one of the most-promising biocontrol agent that can be used against many fungal plant pathogens. Antibiosis is the generally recognized principal mechanism of interference competition by which fungi may exclude other organism from resources potentially available to each other (Gomathy and Ambikapathy 2011). *T.harzianum* has been also reported as biocontrol agent for the control of *A.alternata* (Roco and Perez, 2001; Monte, 2001; Sempere and Santamarina, 2007). In the present study *Trichoderma* spp. overgrew the pathogenic fungus. Mycoparasitism was caused by *T.harzianum* ISO-1, *T.harzianum* ISO-2 and *T.piluliferum* through physical contact which resulted in coiling and cell lysis in *A.alternata*.

Elad *et al*. (1982) reported that lysis and disintegration of mycelium of test fungus may be due to action of enzymes produced by *Trichoderma* spp.

Kumar (2008); Gveroska and Ziberoski (2012); Rajput *et al*. (2013) studied *in vitro* antagonistic efficacy of *Trichoderma harzianum* on the growth of *Alternaria alternata* by dual culture method.

Similar findings were made by Balai and Ahir (2011) that the bioagent *T. harzianum* was found to be most effective in inhibiting the growth of *A.alternata* followed by *A.niger*. Ambuse *et al*. (2012) tested three species *Trichoderma* viz., *T. viride*, *T. koningii* and *T. pseudokoningii* against *Alternaria tenuissima* and found 80% antagonistic activity of *Trichoderma* sp. against *Alternaria tenuissima*. Jat and Agalave (2013) reported the antagonistic properties of *Trichoderma* sp. against *Alternaria alternata*. Panwar *et al*. (2013) studied the efficiency of *Aspergillus niger* in inhibiting the radial growth of *A.alternata* through dual culture technique.

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