Phylloplane microflora as foliar biocontrol agents against Leaf spot of *Baccopa monniere*

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Phylloplane fungi presented on the surface of leaf were screened and selected for the evaluation of their potential against *Alternaria alternata* causing Leaf spot disease of *Baccopa monniere* . *In vitro* studies conducted to evaluate the efficacy of phylloplane fungi. *Trichoderma harzianum* ISO-1, *T.harzianum* ISO-2 and *T.piluliferum* and it was found that all these fungi caused maximum inhibition of test pathogen followed *Aspergillus niger* and *Penicillium sublateritium* whereas *P. tardum* and Cladosporium cladosporioides showed minimum antagonistic efficacy.

Key words: Biocontrol, antagonistic efficacy, phylloplane fungi, *Baccopa monniere*

INTRODUCTION

The application of biological control of plant diseases is a very prospective method for keeping an environment clean and healthy. Biological control of pathogens accounts on the application of microorganisms which inhibits or suppresses the causal pathogenic agent. A biochemical basis in antagonistic relations among microorganisms is very interesting in a present time, to aim at developing new fungicides.

Plant diseases are mostly controlled by the use of chemical pesticides and in some cases by cultural practices. However, the widespread use of chemicals in agriculture has been a subject of public concern and scrutiny due to the potential harmful effects on the environment, their undesirable effects on non-target organisms and possible carcinogenicity of some chemicals.

Biodogical Control Agents (BCAs) interact with plant and pathogens that supress the growth of pathogen growth by direct and indirect mechanisms involved in the working of antagonist including hyperparasitism, competition, induction of host resistance, production of antibiotics (Phenazines, 2.4-diacetyl pholroglucinol), lytic enzymes (Chitinases, proteases, glucanases), non-regulatory waste products (hydrogen cyanide, ammonia, carbon dioxide) that lead to biocontrol of plant parasitic pathogens .

Thakur and Harsh (2014),have reported that phylloplane fungi show potential as a biocontrol agent against Alternaria leaf spot disease of Spilanthes oleraceae. Biological control using fungal and bacterial antagonists to manage plant diseases seems to be a promising alternative strategy and have successfully been applied to control some diseases on different plants and crops.

Heydari and Mohammed (2010) have reported that biocontrol strategies may also be used to manage other plant diseases including foliar ones. Some of the important factors that affect the efficacy of microbial biocontrol agents in controlling plant diseases which should carefully be considered include method of application, formulation of biocontrol microorganisms and timing of application.

Baccopa monniere Linn. belonging to the family Plantagenaceae is a small succulent herb with numerous branches and rooting at the nodes, it is a therapeutic herb commonly known Brahmi and used as a memory enhancer. Brahmi can be a great remedy that provides relief from arthritis gout and other inflammatory conditions. B.monniere is attacked by Alternaria alternata

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Keissler causing leaf spot disease resulting into failure of crop (Bhandari *et al*.2014).The antagonistic efficacy of *Trichoderma harzianum* and *Aspergillus niger* against *Alternaria brassicae* isolated from phylloplane of mustard. Has been analyzed by Reshu and Khan (2012), Thakur and Harsh (2014) have reported that *Trichoderma harzianum* effectively inhibited the growth of pathogenic fungi in the dual culture. The present study shows phylloplane fungi for their antagonistic efficacy against Alternaria leaf spot of *B. monniere.*

MATERIALS AND METHODS

Isolation of leaf pathogen

Leaves of *B.monniere* infected with *A.alternata* were collected from Non Wood Forest Products Division Nursery, Forest Research Institute, Dehradun and Uttarakhand. Isolation of pure culture of fungal pathogen was done by taking a portion of leaf (which is containing brown spot) 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 *B.monniere* through leaf washing technique 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.

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 diameter 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±1ºC 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±1ºC 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.

Antagonistic efficacy = b + c – a where,
a = % of area of test pathogen sp. with antagonist in the same Petri plate (cm^3)
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

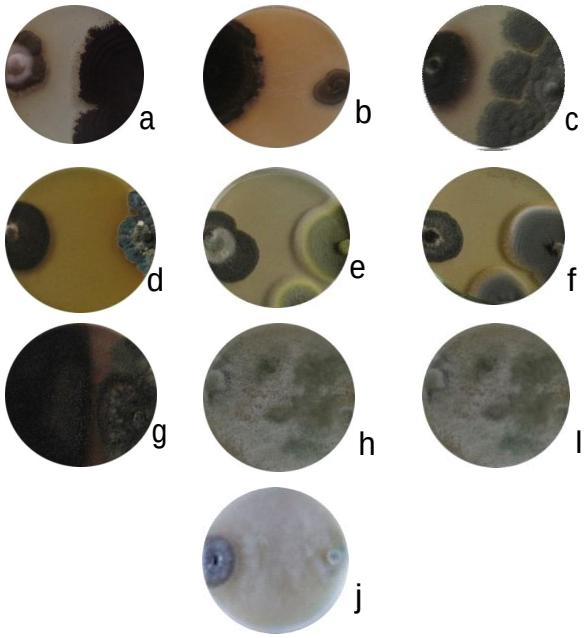

Fig. 1 : Screening of antagonistic fungi against *A.alternata* (R.s) a) *A.nig-er*,b)*C.cladosporioides*,c)*P.citreo-viride*,d) *P.frequentans*,e)*P.herquei*,f)*P.sublateritium*,g)*P.tar-dum*,h)*T.ha-rzianum* ISO-1, i)*T.harzianum* ISO-2,j)*T.piluliferum*

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 after inoculation and per cent 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 after inoculation and percent inhibition of *A.alternata* are summarized in Table 1.

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

Antagonists	Percent Antagonistic efficacy (Mean±S.D.)
A. niger	67.40(85.07)±3.44
C. cladosporioides	27.57(21.43)±0.79
P. citreo-viride	63.39(79.93)±0.70
P. frequentans	37.37(37.07)±7.17
P. herquei	55.28(67.47)±3.75
P. sublateritium	64.13(80.93)±1.34
P. tardum	24.05(16.67)±2.00
T. harzianum ISO-1	90.00(100)±0.00
T.harzianum ISO-2	90.00(100)±0.00
T.plililiferum	90.00(100)±0.00
Mean	60.92
SEM±	1.67
CD at 5%	4.94

*Original values are given in parentheses

Trichoderma harzianum ISO-1, *T.harzianum* ISO-2 and *T.piluliferum* inhibited the growth of *A.alternata* by 90.00% and exhibited maximum efficacy followed by *A.niger* (67.40%) and *P.sublateritium* (64.13%). Antagonists *P. citreo-viride* (63.39%), *P. herquei* (55.28%) and *Trichoderma harzianum* ISO-1, *T.harzianum* ISO-2 and *T.piluliferum* inhibited the growth of *A.alternata* by 90.00% and exhibited maximum efficacy followed by *A.niger* (67.40%) and P.sublateritium (64.13%). Antagonists *P. citreo-viride* (63.39%), *P. herquei* (55.28%) and *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).Similar report have been published earlier by Panwar *et al*. (2013) and Rajput *et al.* (2013)

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