Research Paper

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Phylloplane Mycoflora as Antagonist to *Alternaria alternata* causing leaf spot of *Stevia rebaudiana*

Botany

KEYWORDS : *Alternaria alternata; Stevia rebaudiana*; phylloplane fungi

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ABSTRACT

Leaf spot disease (Alternaria alternata) is one of most yield limiting constraints of Stevia rebaudiana Bertoni. The objective of this study was to isolate and evaluate antagonistic potential of phylloplane fungi against the pathogen in controlling the leaf spot disease. Qualitative studies on mycoflora showed the presence of 20 fungi belonging to 11 genera with sterile form and pink yeast. Interaction of pathogen with phylloplane fungi was determined by dual culture. Penicillium expansum caused maximum inhibition (75.11%) of test pathogen followed by Trichophyton sp. (71.15%), Aspergillus niger (65.96%), A. awamori (53.57%), A. flavus (48.0%), Penicillium purpurogenum (47.50%), Cladosporium cladosporioides (43.48%), Penicillium cyclopium (40.90%), Periconiella sp. (36.74%), Aspergillus sp. (36.21%), A. fumigatus (33.33%), Curvularia lunata (33%), Penicillium oxalicum (30.08%), P. notatum (26.0%) and Sporothrix sp. (20.83%). Best antagonistic fungi can be used as possible biocontrol agents.

INTRODUCTION

Stevia rebaudiana is a sweet perennial herb, used as a low-caloric sweetener i.e., as a sugar substitute (Geuns 2003). Among the therapeutic activities of *S. rebaudiana* some important are antidiabetic, hypoglycemic, antiobesity, plague retardant, skin toning and healing burns and wounds (Maiti & Purohit, 2008).

This commercially and medicinally important plant endures a leaf spot disease caused by the fungus *A. alternata* in various parts of India. This leading to significant quantitative and qualitative loss of *Stevia* leaves.

A large number of synthetic inorganic and organic fungicides have been developed to control plant diseases, also shows residual toxicities (Sharma *et al*., 2004). Biological control is another option, which is potential, alternative, ecofriendly, self-perpetuating usually free from residual effects and also can be an important component of integrated disease management (Sahile *et al*., 2011). Strains of microorganisms to be used as biocontrol agents are ideally best obtained from the same foliar environment as the pathogen which they are required to control and provided sufficient activity against the pathogens (Blakeman *et al*., 1992).

In this study we have isolated phylloplane mycoflora to evaluate their *in vitro* fungitoxicity against the phytopathogenic fungi *A. alternata*.

MATERIAL AND METHODS

Isolation of phylloplane fungi

Young and mature healthy leaves of *S. rebaudiana* were collected separately in sterile boxes from Sushila Tiwari Herbal Garden, Rishikesh. Fungi were isolated by using leaf washing method (Kumar & Singh, 1981).

$$\text{Fungal density} = \frac{\text{No.of spores in 1 ml}}{\text{Weight of leaves (g)}} \times \text{Dilution factor}$$

Isolation of pathogen

Infected leaves of *S. rebaudiana* were collected and infected parts were cut with blade. To avoid phylloplane microflora, the surface of infected parts was sterilized and placed on Sabouraud dextrose agar medium. The plates were incubated at 25°C±2°C until full-fledged fungi appeared. Pathogenicity test was carried out to confirm that the isolated organism was actually a pathogen.

Identification of Fungi

All the fungi were identified on the basis of their cultural and microscopic characteristics by using taxonomic keys (Ellis 1971, 1976; Raper & Thom 1949; Thom & Raper 1945; Barnett & Hunter 1972) as *A. alternata, Aspergillus fumigatus, A. awamori, A. flavus, A. niger, Aspergillus* sp., *Botrytis cinerea, Cladospoium cladosporioides, Curvularia lunata, Histoplasm*, *Penicillium notatum, P. purpurogenum*, *P. expensum, P. oxalicum, P. cyclopium, Periconiella* sp., *Scytalidium lignicola*, *Scytalidium* sp., *Sporothrix* sp., *Stachybotrys*, *Trichophyton* sp., pink yeast and sterile fungi.

In-vitro Screening of Fungal Antagonists

The interfungal interaction between phylloplane fungi and pathogen was done by Dual culture method. The mode of interaction between pathogen and antagonists were categorised as followed by Skidmore & Dickinson (1976).

0: No visible sign of inhibition: Pathogenic fungi overgrew the test organism

1: Mutual inhibitions: Both organisms stopped growing on contact

2: Inhibition of pathogen with inhibition zone > 1cm in width

3: Inhibition of pathogen with inhibition zone < 1cm in width

4: Inhibition of pathogen by overgrowth

Percentage inhibition of radial growth was assessed using the following two parameters (Fokkema, 1973).

(i) Percentage of growth inhibition

$I = (r - r')/r \times 100$

r= growth of pathogen unopposed by antagonist

r'= growth of pathogen opposed by antagonist

(ii) Width of inhibition zone

RESULTS

Qualitative and quantitative data of the fungi are shown in Table-1. Dual culture studies for evaluation of antagonistic activity of phylloplane fungi revealed that only 15 species of the fungi inhibited growth of *A. alternata* by degrees. Rest seven fungi did not show antagonistic activity against *A. alternata* and rated 0. Percentage inhibition of *A. alternata* by antagonistic fungi and measurement of inhibition zone are represented as bar diagram

in Figure-2. *Trichophyton* sp. grew about 2 cm over the colony of *A. alternata*. It started grew over pathogen at sixth day of incubation and inhibited its growth by 71.15%. The interaction was rated 4. *P. expansum* inhibited the growth of *A. alternata* by 75.11% through antibiosis and interaction was rated 2. *C. lunata*, *Sporothrix* sp. and *C. cladosporioides* inhibited the growth of *A. alternata* by 33%, 20.83% and 43.48% respectively and stopped growing at the point where they came in contact with *A. alternata*. These all was rated 1. *Periconiella* sp. grew 2 mm over mycelium of *A. alternata* and inhibited its growth by 36.74% and rated 4. We observed that *Periconiella* sp. overgrew *A. alternata* at 10th day of incubation. Most of *Aspergillus* sp. showed mutual inhibition. Only one species of *Aspergillus* produced 6 mm inhibition zone against *A. alternata. A. awamori*, *A. flavus*, *Aspergillus* sp. and *A. fumigatus* inhibited the growth of *A. alternata* by 53.51%, 48.0%, 36.21% and 33.3% respectively. *A. awamori, A. flavus* and *A. fumigatus* were rated 1 while *Aspergillus* sp. rated 3. *A. niger* overgrew 4 mm colony of *A. alternata* on seventh day of incubation and inhibited its growth by 65.96% which was rated 4. All *Penicillium* species produced 2-11 mm inhibition zones. *P. notatum, P. purpurogenum, P. oxalicum and P. cyclopium* inhibited the growth of *A. alternata* by 26%, 47.5%, 30.08% and 40.9 respectively. *P. notatum* and *P. cyclopium* were rated 3 and *P. oxalicum* and *P. purpurogenum* rated 2. Except *Trichophyton* sp., *Periconiella* sp. and *A. niger* all the fungi did not overgrow *A. alternata* till the end of the experiment. Panwar *et al.* (2013) studied efficiency of *A.niger* in inhibiting the radial growth of *A. alternata* through dual culture technique. Rajput *et al.*, (2013) screened *Trichoderma viride*, *T. harzianum, Aspergillus niger, Gliocladium virens, Chaetomium globosum* against *A. alternata* and found that *T. viride* showed maximum inhibition followed by *T. harzianum, G. virens, A. niger* and least by *C. globosum*.

Microscopic observations of mycelial interactions

Interaction of *A. alternata* with *Trichophyton* sp., *Periconiella* sp. and *A. niger* was observed microscopically. Mycoparasitism was caused by *A. niger* through physical contact which resulted in coiling and cell lysis in *A. alternata* and the pathogen could not be re-isolated from the point of contact. Mycelium of *Trichophyton* sp. caused coiling of mycelium and conidia in *A. alternata*. *Periconiella* sp. did not show any clear pattern of hyphal interaction.

DISCUSSION

Hostile effects of different phylloplane fungi to *A. alternata* indicated role of many such fungi as a possible biocontrol agent. *Trichophyton* sp. and *A. niger* antagonized *A. alternata* by overgrowth mechanism. This was due to competition between saprophytic and pathogenic microorganisms for nutrients. *P. expensum*, *Trichophyton* sp., *A. niger* and *A. awamori* showed significant reduction in the growth of *A. alternata*. Interaction between fungal colonies of *A. alternata* with all *Penicillium* sp. and one *Aspergillus* sp. illustrated that they did not intermingled in culture. However, *A. alternata* was inhibited and this is as a result of the production of metabolites, possibly antibiotics by *Penicillium* spp. and *Aspergillus* sp. in the medium. *C. cladosporioides, A. flavus, Aspergillus* sp. *Periconiella* sp., *P. purpurogenum* and *P. cyclopium* showed appreciable reduction in the growth of *A. alternata*. *C. lunata*, *A. fumigatus, Sporothrix* sp., *P. oxalicum* and *P. notatum* showed comparatively least inhibition.

CONCLUSION

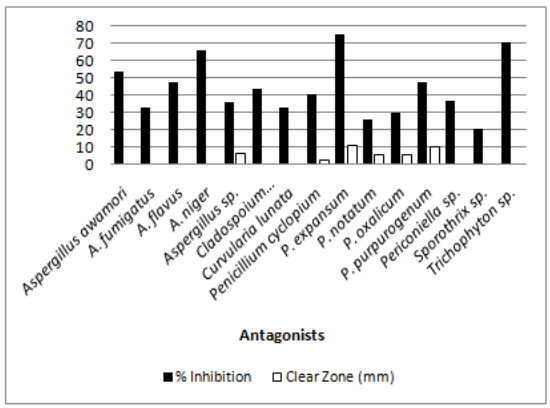
Best antagonists can be suggested as biocontrol agents after pot and field trial against *A. alternata* for management of *S. rebaudiana* leaf spot disease.

Table-1 Density (/gm) of fungal species at 10^{-3} dilution of Young (Y) and Mature (M) leaves

S.N.	*Fungi*	March, 2013		June, 2013		Sept., 2013	
		Y	M	Y	M	Y	M
1.	*Aspergillus awamori*	0.10	0.07	0.07	-	-	-
2.	*A. flavus*	0.07	0.15	0.25	0.3	0.15	-
3.	*A. fumigatus*	-	-	1.15	0.6	-	0.05
4.	*A. niger*	-	0.17	-	-	-	0.07
5.	*Aspergillus sp.*	-	-	0.05	-	-	-
6.	*Botrytis cinerea*	-	-	0.05	-	0.15	-
7.	*Cladosporium cladosporioide*s	0.17	0.07	-	-	0.20	0.05
8.	*Curvularia lunata*	-	-	-	-	0.20	0.05
9.	*Histoplasm* sp.	-	-	-	-	-	0.02
10.	*Penicillium cyclopium*	0.07	0.17	0.8	1.1	-	-
11.	*P. expensum*	0.13	-	-	-	-	-
12.	*P. notatum*	-	-	-	-	0.05	0.20
13.	*P. oxalicum*	-	-	-	-	0.05	-
14.	*P. purpurogenum*	0.10	-	0.25	0.45	-	-
15.	*Periconiella* sp.	0.13	0.13	0.25	0.25	-	-
16.	*Scytalidium lignicola*	-	-	0.03	-	-	-
17.	*Scytalidium* sp.	-	-	0.05	-	0.20	-
18.	*Sporothrix* sp.	-	-	-	-	-	0.05
19.	*Stachybotrys* sp.	-	0.13	-	-	-	-
20.	*Trichophyton sp.*	-	-	-	-	-	0.09
21.	*Pink Yeast*	-	-	0.25	0.65	0.15	0.02

22.	*Sterile form*	0.07	0.10	-	-	-	0.03

Fig.-1 Presentation of clear zone and percentage mycelial growth inhibition of *A. alternata* by Phylloplane fungi



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