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EFFECT OF VOLATILE METABOLITES OF PHYLLOPLANE FUNGI OF *CHLOROPHYTUM TUBEROSUM* AGAINST ITS FUNGAL PLANT PATHOGEN *IN-VITRO*

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ABSTRACT

Phylloplane fungi of *Chlorophytum tuberosum* were screened for their antagonistic activities against *Colletotrichum dematium in vitro*. Volatiles produced from the cultures of *Trichoderma harzianum* ISO-1 showed maximum inhibition of mycelial growth of *C.dematium* followed by *T. piluliferum*, *T. harzianum* ISO-2 and *Aspergillus niger*, respectively.

KEYWORDS: **Volatiles, *Colletotrichum dematium*, *Chlorophytum tuberosum*, Phylloplane fungi**

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INTRODUCTION

Aerial plant surface is considered as a dynamic environment. Therefore, provides a suitable habitat for certain epiphytic microorganisms, the growth and activity of which are highly influenced by the nutrients present on leaf surfaces[1]. Phyllosphere colonization involves development of both pathogen and antagonist in the phyllosphere which is determined by several abiotic factors such as availability of nutrients, leaf exudates, temperature, water, relative humidity, atmospheric gases, light and radiations, wind and pollution[2].Fungicides are widely used to eradicate pathogens but it results in environmental hazards and has harmful side effects besides chemical fungicides also develop fungicide resistant pathogens. In order to overcome such hazards alternative methods such as the use of biological control agents (BCAs) to control fungal plant diseases is being adopted. These days, biological control for plant diseases is now receiving increasing attention, although the potential of biocontrol through the effect of phyllosphere antagonists has been realized recently. [3,4]had explored the ability of certain antagonists fungi for the possible control of pathogenic fungi on aerial plant surfaces.*Chlorophytum tuberosum* Baker belonging to the family Liliaceae is a traditional medicinal plant found in central India and in Deccan peninsula[5]. It is commonly known as *Safed musli*. It contains about 25 alkaloids, vitamins, proteins, carbohydrates, steroids, saponins, etc. Due to its great therapeutic properties “Safed musli” has been valued as a tonic in the treatment of ailments such as diabetes, arthritis, rheumatism and also as an aphrodisiac agent[6]. *C.tuberosum* is attacked by *Colletotrichum dematium* (Pers.) Fr. causing leaf spot disease[7].The present study was carried out to examine the efficacy of volatile metabolites produced by phylloplane fungi against *Colletotrichum dematium* under *in vitro* conditions.

MATERIALS AND METHODS

Isolation of leaf pathogen

Leaves of *C.tuberosum* infected with *C. dematium* were collected from Non Wood Forest Product Division, F.R.I., Dehradun. 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 sterile 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 *C.tuberosum* through leaf washing technique[8,9] 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 for further studies.

Effect of volatile compounds from antagonist(s) on the radial growth of C.dematium

The method described by [10], 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±1°C 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 *C.dematium* and sealed together with cello-tape and re-incubated at 25± 1°C. For control, the lids of uninoculated Petri dishes containing PDA medium were sealed in the same way with bottoms of Petri dishes containing the test pathogen. Radial growth of *C. dematium* was measured after 48, 72 and 96 h. The growth inhibition (%) of the

pathogen was calculated by the the following formula:

Per cent growth inhibition = (C-T) / C×100,

Where, C = Growth in control

T= Growth in treatment

RESULTS AND DISCUSSION

Ten phylloplane fungi were identified 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, *Cladosporium cladosporioides* (Fresen.) de Vries. The phylloplane fungi exhibited varying growth inhibition of *C. dematium* when exposed to their volatile metabolites (Fig.1). All the *Trichoderma* isolates produced volatile metabolites having a significant effect in reducing the radial growth of test pathogen (Table 1). Metabolites produced by *Trichoderma harzianum* ISO-1 was found most effective in reducing the mycelial growth of *C. dematium* by (39.3%) followed by *T. piluliferum* (33.0%), *T. harzianum* ISO-2 (31.3%) and *A. niger* (30.5%). Percent growth inhibition shown by *P. solitum* (22.8%) was at par with *P.citereo viride* (22.4%) and *P. herquei* (17.6%) was found to be at par with *P. sublateritium* (17.2%) respectively (Table1). *P. frequentans* showed mycelial growth inhibition by (13.9%). Whereas, *P. tardum* (5.7%) and *C. cladosporioides* (3.9%) comparatively were least effective in checking the growth of the pathogen.*Trichoderma* spp. have been demonstrated *in vitro* to act against fungal plant pathogens by producing diffusible volatile antibiotics.[11]reported antifungal properties of volatile compounds (Alkyl *pyrones*) produced by *T. harzianum*. Volatile compounds produced from *Trichoderma* species were able to arrest and inhibit the hyphal growth of various plant pathogenic fungi[12]. [13]reported the effectiveness of volatiles produced by *T. harzianum* (Th-1) causing 53.63% inhibition of *Colletotrichum capsici*.

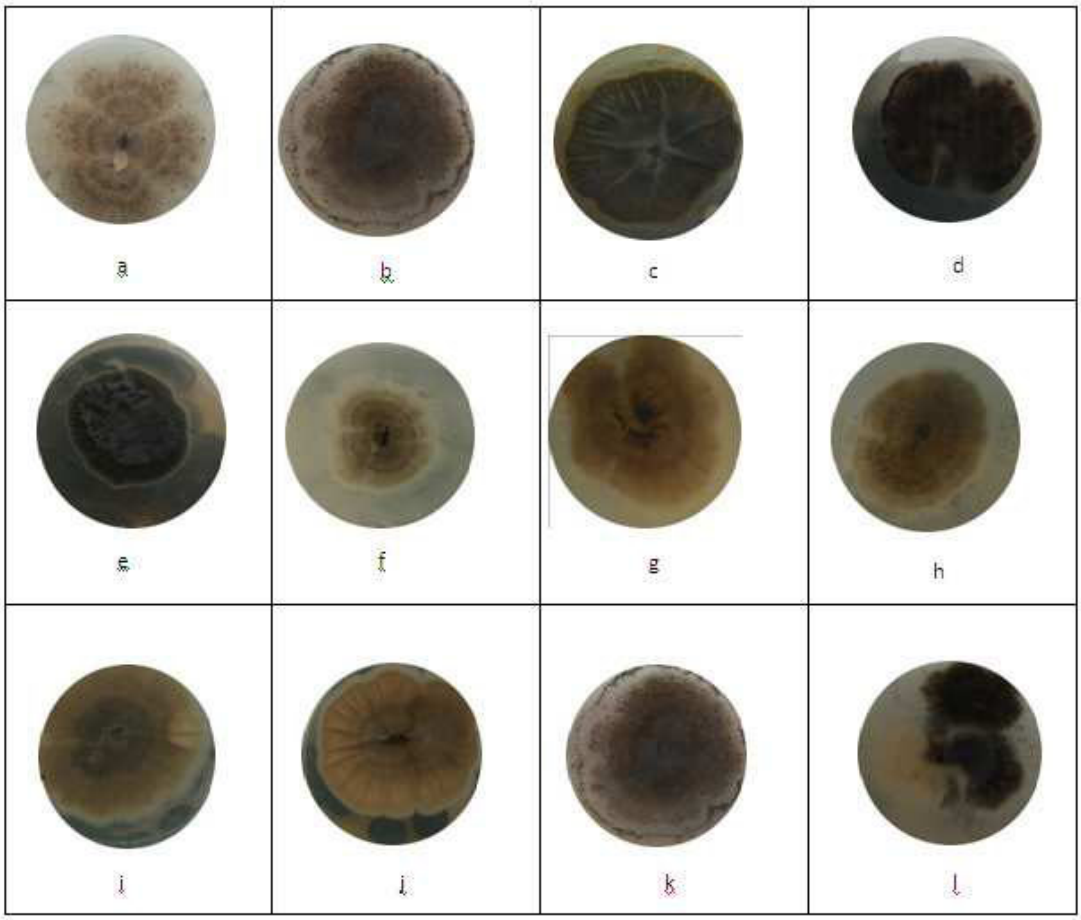

Figure 1

Radial growth of *Colletotrichum dematium* affected by volatile products released from different phylloplane fungi in Petri plates a) Control, b) *P. tardum*, c) *P.herquei*, d) *P. citreoviride*, e) *A. niger*, f) *T. harzianum* ISO-1, g) *T. harzianum* ISO-2, h) *T. piluliferum*, i) *P. sublateritium*, j) *P. frequentans*, k) *C. Cladosporioides*, l) *P. solitum*.

Table 1

Evaluation of volatile metabolites produced by phylloplane fungi against the test pathogen C.dematium

S.no.	Antagonist fungi	% growth Inhibition of *C.dematium* ±S.D.
1.	*P. citreoviride*	22.4±2.40
2.	*P. frequentans*	13.9±0.36
3.	*P. herquei*	17.6±2.45
4.	*P. solitum*	22.8±0.80
5.	*P. tardum*	5.7±1.13
6.	*P. sublateritium*	17.2±1.65
7.	*A. niger*	30.5±4.82
8.	*C. cladosporioides*	3.9±0.30
9.	*T. harzianum* ISO-1	39.3±2.67
10.	*T. harzianum* ISO-2	31.3±3.13
11.	*T. piluliferum*	33.0±5.43
CD at 5%		4.72

CONCLUSION

From the study it can be concluded that *Trichoderma* spp. and *A. niger* were effective in inhibiting the mycelial growth of *C. dematium in vitro* by producing volatile metabolites. Hence they can be used in the field against the pathogen for management of leaf spot disease in *Chlorophytum tuberosum.*

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