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IN VITRO POTENTIAL OF VOLATILE METABOLITES OF PHYLLOPLANE FUNGI OF *PIPER LONGUM* AS BIOCONTROL AGENT AGAINST PLANT PATHOGEN

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ABSTRACT
Fungal phytopathogens are crucial in causing several diseases and imposing significant economic losses. Foliar diseases are of utmost importance as they adversely affect the growth of the plant. The present study was aimed to screened the phylloplane fungi of *Piper longum* for their antagonistic activities against *Colletotrichum gloeosporioides in vitro*. Total eleven fungi were isolated from phylloplane of healthy plants.Volatiles produced from the culture of *Aspergillus niger* showed maximum inhibition of mycelial growth of *C.gloeosporioides* followed by *Trichoderma harzianum* ISO-1, *T.harzianum* ISO-2, *Penicillium sublateritium* and *Trichoderma piluliferum* respectively.

KEY WORDS: Volatiles, *Colletotrichum gloeosporioides*, *Piper longum*, Phylloplane fungi

INTRODUCTION

Last (1955) introduced term phyllospere to denote the leaf surface of the plants. 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. Biological components of phylloplane are affected by leaf exudates, fluctuating temperature, relative humidity, atmospheric gases, light and radiations,wind and pollution (Lindow, 2006). Therefore, aerial plant surface is considered as a dynamic environment (Fokkema,1976).Fungicides are widely used to eradicate pathogens but their application 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 use of biological control agents (BCAs) to control fungal plant diseases is being adopted. Biological approaches for the control of pathogens on aerial surfaces have been reviewed extensively (Andrew, 1990 & 1992; Blakeman and Fokkema, 1982; Elad, 1993; Fokkema, 1993; Spurr and Knudsen, 1985). Thus, biocontrol offers an attractive alternatives or supplements to the use of conventional method of disease control (Compant *et al*., 2005). Euveh *et al*. (2008 & 2011) had explored the ability of certain antagonistic fungi for the possible control of pathogenic fungi on aerial plant surfaces. *Piper longum* Linn. belonging to the family Piperaceae is a slender aromatic climber with perennial woody roots. It grows all over India in evergreen forests and is cultivated in Assam, Tamil Nadu and Andhra Pradesh (Anon., 1969). It has been valued as a tonic in the treatment of bronchitis, asthma, respiratory tract diseases; as cholagogue, in bile, gall bladder obstruction, and as a sedative in insomnia and epilepsy (Dahanukar *et al*., 1984). *P. longum* is attacked by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. causing leaf spot disease (Puni and Harsh, 2009). The present study was carried out to examine the efficacy of volatile metabolites produced by phylloplane fungi against *Colletotrichum gloeosporioides* under *in vitro* conditions.

MATERIALS & METHODS

Isolation of leaf pathogen

Leaves of *P.longum* infected with *C. gloeosporioides* were collected from Biotech Green Herbal and Medicinal Plant Nursery, Kolhupani, Dehradun, 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 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 *P.longum* through leaf washing technique (Dickinson, 1967; Aneja, 2003) 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 for further studies.

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

The method described by Dennis and Webster (1971) 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 disc 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 disc of the *C. gloeosporioides* 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. gloeosporioides* was measured after 48, 72 and 96 h. The growth inhibition (%) of the pathogen was calculated by the following formula:

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

Where, C = Growth in control

T = Growth in treatment

RESULTS

Eleven 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.solitum* Westling, *P.tardum* Thom, *P. citreo-viride* Biourge and *Cladosporium cladosporioides* (Fresen.) de Vries. The phylloplane fungi exhibited varying growth inhibition of *C.gloeosporioides* when exposed to their volatile metabolites (Fig.1).

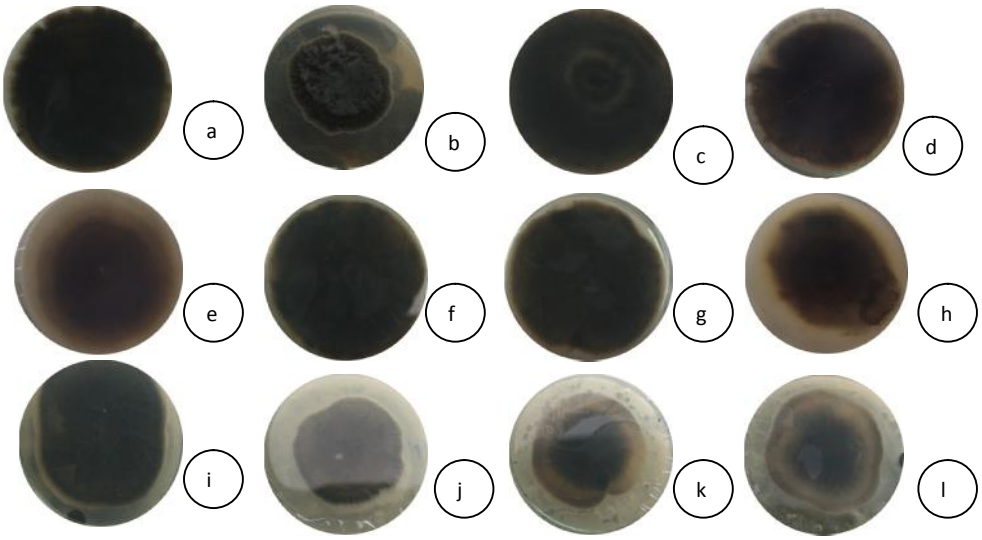

FIGURE 1. Radial growth of *Colletotrichum gloeosporioides* affected by volatile products released from different phylloplane fungi in Petri plates a)Control, b)*A. niger*, c)*C. cladosporioides*, d)*P. frequentans*, e)*P. citreoviride*, f)*P. herquei*, g)*P. solitum*, h) *P. sublateritium* i)*P. tardum*,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

Results showed that of the eleven potential antagonists *A. niger* (42.43%) proved to be the most effective biocontrol agent in inhibiting mycelial growth of *C. gloeosporioides* (Table 1). All the *Trichoderma* isolates produced volatile metabolites having significant effect in reducing the radial growth of the test pathogen. *Trichoderma* isolates viz., *Trichoderma harzianum* ISO-1 reduced the mycelial growth of test pathogen by 38.96%. Percent growth inhibition shown by *T.harzianum* ISO-2 (36.40%) was at par with *P.sublateritium* (36.93%).Whereas, *T. piluliferum* showed 31.26% inhibition. Metabolites produced by *P.tardum* (17.60%), *P.citreo viride* (13.90%) and *P.frequentans* (11.23%) showed moderate level of inhibition. *P.herquei* (6.83%), *P.solitum* (4.70%) and *C.cladosporioides* (4.70%) comparatively were least effective in checking the growth of the pathogen.

TABLE 1. Evaluation of volatile metabolites produced by phylloplane fungi against the test pathogen *C.gloeosporioides*

S.No.	Antagonist	Per cent inhibition of mycelial growth (Mean ± S.D.)
1.	*Aspergillus niger*	42.43 ± 2.05
2.	*Cladosporium cladosporioides*	4.70 ± 0.10
3.	*Penicillium frequentans*	11.23 ± 0.61
4.	*Penicillium citreoviride*	13.90 ± 0.36
5.	*Penicillium herquei*	6.83 ± 0.92
6.	*Penicillium solitum*	4.70 ± 0.10
7.	*Penicillium sublateritium*	36.93 ± 2.87
8.	*Penicillium tardum*	17.60 ± 2.45
9.	*Trichoderma harzianum* ISO-1	38.96 ± 1.83
10.	*Trichoderma harzianum* ISO-2	36.40 ± 3.13
11.	*Trichoderma piluliferum*	31.26 ± 3.13
Mean		22.27
SEM ±		1.14
CD at 5%		3.34

DISCUSSION

Pandey *et al*. (1993) have also reported that the volatiles produced from *A. niger* effectively inhibited the growth of *C. gloeosporioides*. *Trichoderma* spp. has been demonstrated *in vitro* to act against fungal plant pathogens by producing diffusible volatile antibiotics. Claydon *et al*. (1987) reported antifungal properties of volatile compounds (Alkyl *pyrones*) produced by *T. harzianum*. *Penicillium* spp. were also found capable of producing volatile antibiotics in agar (Jayasuriya *et al*., 1996). Doi and Mori (1994) reported volatile compounds produced from *Trichoderma* species were able to arrest and inhibit the hyphal growth of various plant pathogenic fungi. Ajith and Lakshmedevi (2010) examined the potential of *Trichoderma harzianum* which suppress the mycelial growth of *Colletotrichum capsici* causing anthracnose on bell peppers. Famin *et al*. (2010) reported the effectiveness of volatiles produced by *T.harzianum* (Th-1) causing 53.63 per cent inhibition of *Colletotrichum capsici*.

CONCLUSION

This study demonstrates that *A. niger*, *P. sublateritium* and *Trichoderma* spp. possess higher antagonistic efficacy in inhibiting the mycelial growth of *C. gloeosporioides in vitro* by producing volatile metabolites. Hence the phylloplane fungi can be applied in the field as an alternative to the synthetic fungicides for the management of leaf spot disease in *Piper longum*.

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REFERENCES

Ajith, P.S. and Lakshmidevi. N (2010) Effect of volatile and non-volatile compounds from *Trichoderma spp*. against *Colletotrichum capsici* incitant of anthracnose on bell peppers. Nature and Science. **8(9),** 265-269.

Andrew, J. H. (1990) Biological control in the phyllosphere: Realistic goal or false hope. *Canadian Journal of Plant Pathology*.**12**, 300-307.

Andrews, J. H. (1992) Biological control in the phyllosphere. *Annual Review of Phytopathology*. **30**, 603-635.

Aneja, K.R. (2003) Experiments in Microbiology, Plant Pathology and Biotechnology, in *Isolation of microorganisms from phyllosphere(phylloplane).* 4th (eds.), 176p, New Age International Publications Limited.

Anon., (1969).The Wealth of India. Vol.**VIII,** 96-99.

Blakeman, J.P. and N.J. Fokkema (1982) Potential for biocontrol of plant diseases on the phylloplane. *Annual Review of Phytopathology*. **20**,167-192.

Claydon, N., M.Allan, J.R. Hanson and A.G. Avent (1987) Antifungal alkyl *pyrenes* of *Trichoderma harzianum*. *Transactions of the British Mycological Society*. **88**, 503-513.

Compant, Stephane., Duffy, Brion., Nowak, Jerzy., Clement, Christophe and Barka, Essaid Ait (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action and future perspectives. *Applied and Environmental Microbiology*. **71(9)**, 4951-4959.

Dahanakur, S.A., S.M. Karandikar and M. Desai (1984). Efficacy of *Piper longum* in childhood asthma. *Indian drugs*. **21**, 384-388.

Dennis, C and Webster, J (1971) Antagonistic properties of species groups of *Trichoderma* Production of volatile antibiotics. *Transactions of the British Mycological Society*. **57**, 25-29.

Dickinson, C.H. (1967) Fungal colonization of Pisum leaves. *Canadian Journal of Botany*. **45**, 915-927.

Doi, S. and M. Mori (1994) Antifungal properties of metabolites produced by *Trichoderma* isolates from saw dust media of edible fungi against wood decay fungi. *Material and Organismen*. **28**, 143-151.

Elad, Y. (1993) Microbial suppression of infection by foliar plant pathogens. *IOBC Bulletin*.**16**, 3-7.

Euveh, A.G., J.A. Okhuoya, O.O. Osemwegie, A.I. Hamad and O.N. Ogbebor (2011).Evaluation of phylloplane fungi as biocontrol agent of Corynespora diseases of rubber (*Hevea brasiliensis* Muell.Arg.).*World Journal of Fungal and Plant Biology*. **2(1)**, 1-5.

Evueh, G.A. and N.O. Ogbebor (2008) Use of phylloplane fungi as biocontrol agent against *Collectotrichum* leaf disease of rubber (*Hevea brasiliensis*). *African Journal of Biotechnology*.**7(15)**, 2569-2572.

Fahim, A.,V.K. Razdan, F.A. Mohiddin, K.A. Bhat and P.A. Sheikh (2010) Effect of volatile metabolites of *Trichoderma* species against seven fungal plant pathogens *in-vitro*. *Journal of Phytology*. **2(10)**, 34-37.

Fokkema, N. J. (1976) The role of saprophytic fungi in antagonism against *Drechlera sorokiniana (Helminthosporium sativum)* on agar plates and on rye leaves with pollen, *Physiology Plant Pathology*. **3**, 195-205.

Fokkema, N.J.(1993) Oppurtunities and problems of control of foliar pathogens with microorganisms. *Pestic Sci*. **37**, 411-416.

Jayasuriya, K.E. and J.E. Deacon (1996) Possible role of 2- Furaldehyde in the biological control of white root disease of rubber. *J. Rubber Res. Inst.***77**, 15-77.

Last, F.T. (1955)The spore content of air within and above mildew infested cereal crops. *Trans.Br. Mycol. Appl*. **44**, 289-294.

Lindow, S. (2006) Phyllosphere Microbiology: A Perspective. Microbial Ecology of Aerial Plant Surfaces.pp. 1-20.

Pandey, R.R., D.K. Arora and R.C. Dubey (1993) Antagonistic interactions between fungal pathogens and phylloplane fungi of guava. *Mycopathologia*.**124**, 31-39.

Puni, Lokho and N.S.K. Harsh (2009) Studies on nursery diseases of important medicinal plants of Uttarakhand Non wood Forest Products Division Forest Research Institute (Indian Council of Forestry Research and Education). Dehradun.78p.

Spurr, H.W. and G.R. Knudsen (1985) Biological control of leaf diseases, with bacteria. In: Biological Control on the Phylloplane (CE Windels and SE Lindow, eds), pp 45-62, American Phytopathological Society Press, MN.