J. Mycopathol. Res. 52(1) : 81-85, 2014; (ISSN 0971-3719)

Efficacy of bioagents and biological interaction of *Alternaria solani* with phylloplane mycoflora of tomato

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Received : 15.07.2013 Accepted : 21.01.2014 Published : 28.04.2014

Tomato is one of the most popular and widely grown vegetables in the world ranking second in importance next to potato in many countries. *Trichoderma viride*, *T. harzianum* and *T. virens* were found promising antagonists of *A. solani* under *in vitro* evaluation carried out by dual culture technique for growth inhibition. *Trichoderma* not only overgrew the host fungus *A. solani*, but also revealed mycelial coiling. There was strong reducing effect on the development of *A. solani* with various mechanisms of antagonistic influence. Among the eight phylloplane fungi *viz.*, *Cladosporium* sp., *Fusarium* sp., *Curvularia* sp., *Alternaria alternata*, *Penicillium* sp., *Aspergillus flavus*, *Aspergillus niger* and *Trichoderma* sp. evaluated under *in vitro* conditions against *A. solani* for antagonistic activity, *A. niger*, *Trichoderma* sp., *A. flavus* and *Curvularia* sp. were found highly potential antagonistic in terms of growth inhibition. Antagonistic effects of mycoflora isolated from phylloplane warrant further investigation to expoit effectively against pathogenic fungi.

Key words: Phylloplane, Mycoflora, *Alternaria solani*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is a crop of immense value in olericulture. It is a Solanaceous fruit vegetable originated in tropical America (Thompson and Kelly, 1957). Portuguese introduced it in India in the early 18th century. It is grown both under field as well as green house conditions throughout the year. The estimated area under tomato in India is about 6,33,000 ha and production was 1,24, 25,000 tonnes of fruits. In Gujarat, tomato is grown in an area of 33,800 ha with the production of 8,41,300 tonnes (Anon., 2010). The important tomato growing districts of Gujarat state are Gandhinagar, Ahmadabad, Baroda, Kheda, Sabarkantha, Surat, Valsad, Jamnagar and Junagadh (Anon., 2009).

Tomato has got medicinal value. It is one of the richest vegetables which keeps our stomach and intestine in good condition (Bose and Som, 1986). The fruits are consumed as raw or cooked. In India, Gujarat is one of the major states cultivating tomato crop. This crop is adversely affected by early blight disease and suffers severe losses, both quantitatively and qualitatively (Patel and Chaudhary, 2010).The greatest data in biological control literature, refers to genus *Trichoderma*. The activity of this useful species has been recognized from 1930 and today there are modern technologies for including them in biological control of various diseases. For a long time, *Trichoderma* species have been known as biological agents for control of plant diseases (Ranasingh *et al.*, 2006). They interact with root, soil and leaf surroundings. They produce and release many components, which induce local or systemic plant resistance to abiotic stress. According to Rosado *et al.* (2007), the main factor for ecological success of this genus is a combination of very active mycoparasitism mechanisms and an effective defensive strategy, induced in the plants.All phylloplane mycoflora may not necessar-

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ily be pathogenic to plant. Certain such mycoflora may be antagonist to the pathogenic fungus, which also warrants to be assessed in order to exploit beneficial phylloplane mycoflora, if any.

MATERIALS AND METHODS

Efficacy of bioagents

Antagonism study of five bioagents were carried out by dual culture technique against *A. solani*. Five mm mycelial disc of about seven days old culture of respective antagonist and test pathogen was placed in the Petri plate containing solidified PDA at about seven cm distance. The medium inoculated with the pathogen alone served as control. The plates were incubated at 25± 2 °C. Colony diameter of the test fungus as well as respective antagonist up to the zone of inhibition was recorded. Per cent growth inhibition of pathogen was calculated by the following formula given by Arora and Dwivedi (1979).

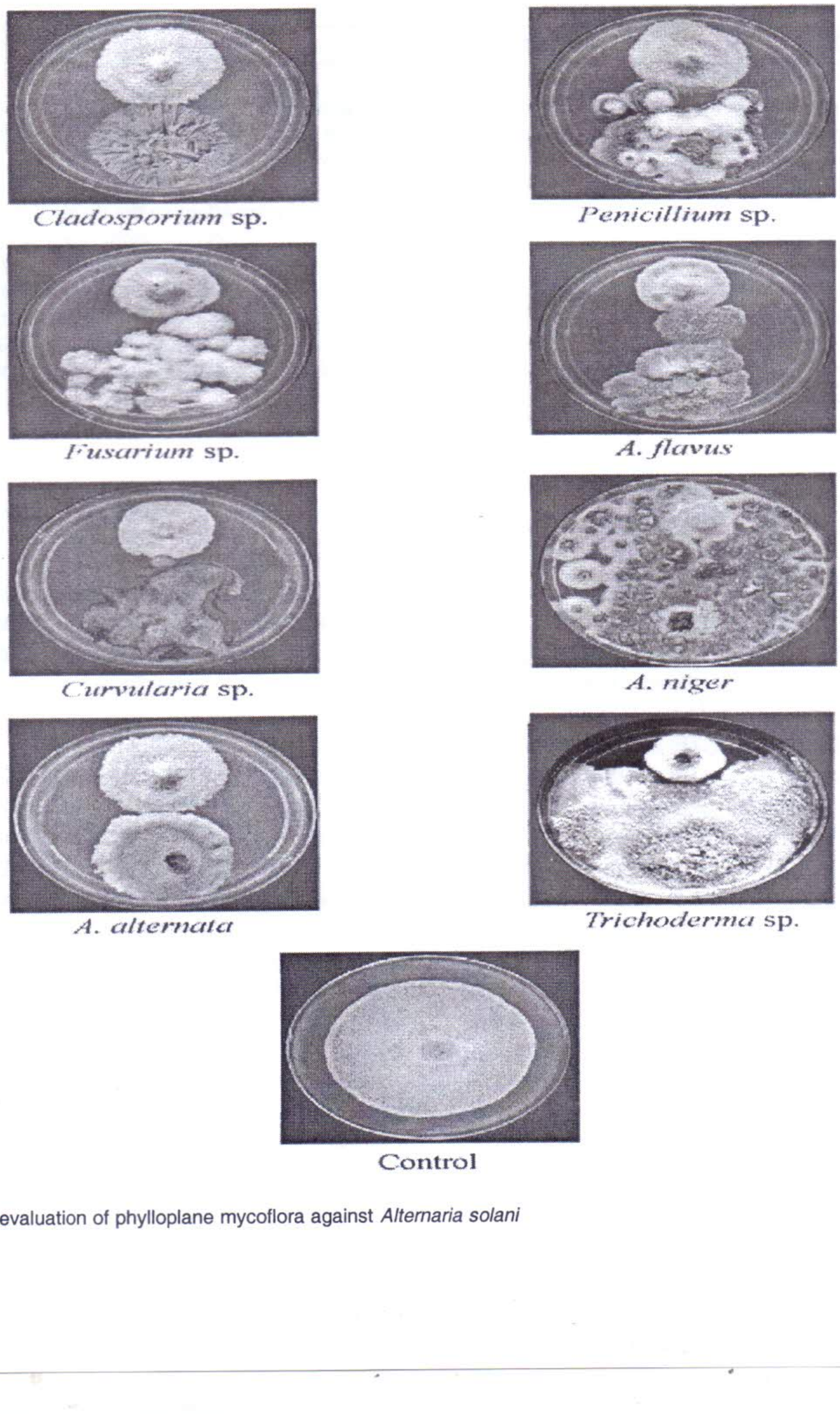


Fig. 1 : *In vitro* evaluation of phylloplane mycoflora against *Alternaria solani*

$$PGI = \frac{DC - DT}{DC} X\ 100$$

where,

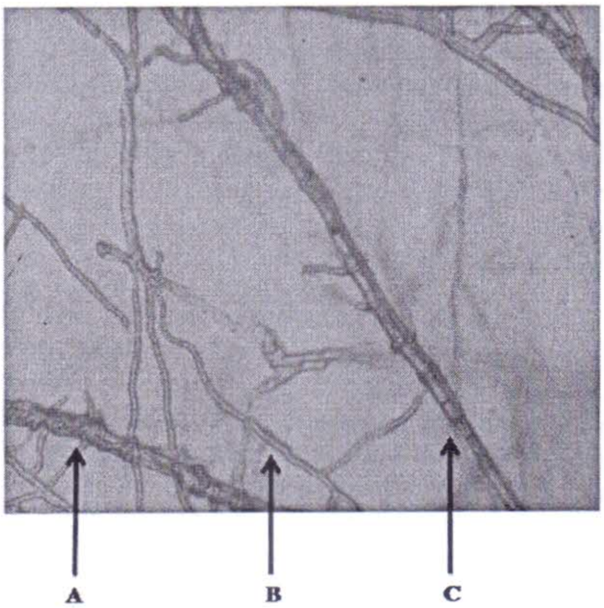
PGI = Per cent growth inhibition
DC = Average diameter (mm) of mycelial colony in control treatment
DT = Average diameter (mm) of mycelial colony in treated set

Isolation of phylloplane mycoflora

Tomato leaves showing *Alternaria* infection were collected from the fields. Isolation from non-surface sterilized leaves was done by dilution plate technique on PDA. PDA was amended with 100 ppm streptomycin sulphate antibiotic just before pouring to avoid bacterial contamination. Colonies of fungi developing on PDA were subcultured and purified by single spore isolation/ hyphal tip method.

In vitro interaction of isolated phylloplane mycoflora against A. solani

The various fungi thus isolated were evaluated under *in vitro* conditions against one representative isolate (AS 1) of *A. solani* for their biological interaction by dual culture method (Ferreira *et al.*, 1991). Five mm mycelial discs of about seven days old culture of respective phylloplane fungus and test pathogen were placed in the Petri plate con-



A : Coiled mycelium of *Alternaria solani*

B : Mycelium of *Trichoderma viride*

C : Septate mycelium of *Alternaria solani*

Fig. 2 : Microphotograph of mycelium of *Alternaria solani* coiled by *T. viride*

Table 1 : Effect of fungal and bacterial bioagents on growth of *A. solani in vitro*

Bioagent	Mycelial Growth (mm)*	Per cent Growth Inhibition (PGI)
Trichoderma harzianum	26.93	66.33
Trichoderma viride	18.90	76.38
Trichoderma virens	27.97	65.04
Pseudomonas fluorescens	34.93	56.33
Bacillus subtilis	40.13	49.13
Control (Untreated)	80.00	–
S.Em.±	1.19	
C.D. at 5%	3.67	
C.V. (%)	5.41	

*Average of three replications

Table 2 : Effect of phylloplane mycoflora on growth of *A. solani in vitro*

Phylloplane Mycoflora	Mycelial Growth (mm)*	Per cent Growth Inhibition (PGI)
Cladosporium sp.	44.07	41.24
Fusarium sp.	30.47	59.38
Curvularia sp.	28.20	62.4
Alternaria alternata	44.53	40.62
Penicillium sp.	31.37	58.17
Aspergillus flavus	26.66	64.44
A. niger	18.50	75.33
Trichoderma sp.	19.27	74.31
Control	75.00	–
S.Em.±	1.08	
C.D. at 5%	3.20	
C.V. (%)	5.29	

*Average of three replications

taining solidified PDA at about seven cm distance. The medium inoculated with the pathogen alone served as control. The plates were incubated at 25± 2°C. Colony diameter of the test fungus as well as respective phylloplane fungus up to the zone of inhibition was recorded, and the per cent growth inhibition of the test pathogen over control, if any, was ealutuated.

RESULTS AND DISCUSSION

Bioefficacy of antagonists against A. solani in vitro

Three fungal bioagents and two bacterial antagonists were evaluated against isolate AS 1 of *A. solani* by dual culture technique *in vitro*. The results presented in Table 1, Fig. 1 revealed that all the antagonists screened against *A. solani* significantly inhibited fungal growth showing 50.00 to 76.38 per cent inhibition over the control. Out of five antagonists, *T. viride* showed significantly maximum per cent growth inhibition (76.38%) with the lowest fungal colony diameter (18.90 mm) of *A. solani* followed by *T. harzianum* (66.33%) with the fungal colony diameter of 26.93 mm. The next best antagonist in order of merit was *T. virens* showing 65.04 per cent inhibition. In case of bacterial antagonists, *P. fluorescens* showed 56.33 per cent growth inhibition followed by *B. subtilis* showing the lowest per cent growth inhibition (49.13%) of *A. solani*.Over all, all the three species of *Trichoderma* revealed higher antagonism as compared to bacterial bioagents against *A. solani*. There was strong reducing effect on the development of *A.solani* with various mechanisms of antagonistic influence. (Fig. 2)

Present findings are in confirmity with earlier research workers. Babu *et al.* (2000) also observed wide inhibition zone of *A. solani* by *T. harzianum* and *T. viride*. Roco and Perez (2001) reported inhibition of *A. alternata* by *T. harzianum*. Arunakumara (2006) found *T. harzianum* effective against *A. solani*. Patel (2007) observed *T. viride* as the most effective antagonistic agent against *A. solani*. Varma *et al.* (2008) reported foliar spray of *T. viride* effective in reducing the early blight disease severity under screen house conditions in tomato. Kumar *et al.* (2010) found *T. viride* effective against *A. solani*.

Biological interaction of phylloplane mycoflora of tomato against A. solani

Eight phylloplane fungi were isolated from tomato leaves. Cultural and morphological characteristics of each of eight fungi studied on PDA plates revealed following characteristics.

Fungus 1

Colonies appeared dark-greenish and revealed slow growth. Reverse was black. The hyphae were found septate. Conidiophores were septate, branched near the apex or middle portion and appeared in cluster or single. Dark conidia were one to two celled, little variable in shape and size. Conidial production was in long branching chain. Conidial formation was in acropetal. Fungus was identified as *Cladosporium* sp.

Fungus 2

Fungus produced white colonies with abundant sporulation. Reverse appeared lavender to purple. Conidiophores were hyaline, very short and bearing conidial masses at the apex. Both macroconidial and microconidial production was abundant. Microconidia were non-septate and slightly curved or straight. Macroconidia were septate and revealed slightly sickle-shaped, thin-walled, with attenuated apical cell and a foot-shaped basal cell. Clamydospores were globose and solitary. Fungus revealed close identity to *Fusarium* sp.

Fungus 3

The colonies of the fungus were black with septate mycelium. Fungus produced erect, brown, simple or branched conidiophores. Conidia were produced both apically and laterally on conidiophores. Sub elliptical and curved conidia revealed mostly three transverse septa. The end cell of each conidium was paler than two central other cells. Fungus was identified as *Curvularia* sp.

Fungus 4

Colonies colour was little dark. Mycelium was septate. Septa were quite conspicuous under microscopic examination. Conidiophores appeared simple or branched, bearing multicelled conidia in chain at the apical part. Conidia were broadest near the base and taper gradually towards the beak. Sometimes, well developed beaks were seen at the tip of the conidia. Fungus was identified as *Alternaria alternata*.

Fungus 5

Fungal colonies were velvety and bright grayish green with whitish tint. Reverse side appeared pale yellowish brown. Microscopic examination revealed dense broom-like spore-bearing structures. Phialides were flask shaped and produced chains of greenish conidia from their tips. Mostly, conidia were globose showing smooth to roughened walls. Fungus was identified as *Penicillium* sp.

Fungus 6

Colonies of the fungus were olive green. The hyphae were septate. Stipes were long and hyaline and revealed little rough wall surface terminated into subglobose vesicles. The vesicles were uniseriate as well as biseriate. Conidia produced in chain were hyaline and one-celled. Fungus was identified as *Aspergillus flavus.*

Fungus 7

Initially, colonies were white which quickly turned black with conidial production. Mycelium growth was quite black and fast growing. It revealed pale yellow colour in reverse side of Petri plates. Mycelium was septate and hyaline. Stipes were long, smooth-walled and terminated into spherical vesicles. Vesicles were biseriate. Conidia were globose and revealed rough surface texture. Fungus was identified as *Aspergillus niger.*

Fungus 8

Fungus developed fast growing green colonies and septate mycelium. Conidiophores were highly branched, loosely or compactly tufted. Conidia were ellipsoidal. All primary and secondary branches of conidiophores arised at or near 90° with respect to the main axis. Typically, the conidiophore terminated in one or a few phialides. Fungus was identified as *Trichoderma* sp.

Biological interaction of phylloplane mycoflora of tomato against A. solani

The eight phylloplane fungi isolated were evaluated under *in vitro* conditions against isolate AS 1 of *A. solani* for their antagonistic activity by dual culture method. The results presented in Table 2; Fig. 1 indicated that all the phylloplane mycoflora have caused a significant reduction of the radial mycelial growth of *A. solani* as compared to control, and proved to be antagonistic to *A. solani* under *in vitro* condition. Out of eight phylloplane mycoflora screened, *Aspergillus niger* showed significantly maximum growth inhibition of 75.33 per cent which was at par with the *Trichoderma* sp. (74.31%) with 18.50 mm and 19.27 mm fungal colony diameter, respectively. The next phylloplane antagonists in order of growth inhibition were *A. flavus* with 64.44 per cent and *Curvularia* sp. (62.4% growth inhibition) with 26.67 mm and 28.20 mm fungal colony diameter, respectively. *Fusarium* sp. recorded 59.38 per cent growth inhibition, and was at par with the *Penicillium* sp. (58.17%). *Cladosporium* sp. and *A. alternata* exhibited moderate growth inhibition of 41.24 and 40.62 per cent of *A. solani.*

Singh and Singh (1997) have reported *Aspergillus terreus*, *Curvularia pallescens*, *Penicillium citrinum*, *Alternaria alternata* and *Trichoderma viride* effective antagonists against *A. solani.* Similarly, the phylloplane fungal isolates *viz.*, *Nigrospora* sp., *Penicillium* sp., *Chaetomium globosum*, *Cladosporium cladosporioides* and *Trichoderma polysporum* have also been reported antagonists to *A. solani* of tomato (Monaco *et al.*, 1999). Sree *et al.* (2007) noted the antagonistic properties of *Cladosporium oxysporum*, *A. niger* and *T. viride* against *A. solani.*

The outcome of the present investigation on antagonistic effects of phylloplane mycoflora warrants further investigation to exploit phylloplane mycoflora effectively against pathogenic fungi.

ACKNOWLEDGEMENTS

The authors are highly grateful to the Professor & Head, Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand for providing the necessary facilities during the course of investigation as well as other faculty members for guidance and moral support during entire research period.

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