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ANTAGONISTIC ACTIVITY OF CULTURAL FILTRATES OF FIVE *TRICHODERMA* SPECIES AGAINST PATHOGENIC FUNGUS *ALTERNARIA SOLANI*

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ABSTRACT

Biological control is a non chemical measure which is usually as effective as chemical control. Several mechanisms are involved in biological control that include, among others, competition for nutrients, induced resistance and secretion of inhibitory substances etc. *Trichoderma* is a soil borne mycoparasitic fungus effective against many soil borne and foliar diseases. *Trichoderma* spp. are often used in agriculture as biocontrol agents against a number of pathogenic fungi *in vitro* and *in vivo*. Culture filtrate of five *Trichoderma* species (viz. *T. viride*, *T. virens*, *T. harzianum*, *T. koningii* and *T. pseudokoningii*) were used against *Alternaria solani.* Culture filtrate of all the species of *Trichoderma* retarded the growth of *A. solani* but *T. viride and T. harzianum* most strongly suppressed the growth of *Alternaria solani*.

Key-words: Culture filtrate, *Trichoderma* species, *Alternaria solani*,

INTRODUCTION

Tomato plants can be affected by several diseases of which one of the most important one is early blight, caused by *Alternaria solani* (Chester, 1950) and it commonly occurs wherever solanaceous crops are grown in the world (Singh, 1983). *A. solani* is an imperfect fungus with no sexual stage known so far. Early blight takes the form of brown leaf spots marked with concentric rings to give the impression of a 'target'. These spots become enlarge slowly and destroy the leaves. The fungus causes stem canker on young seedlings. Infection is accompanied by the production of toxins by *A. solani* called alternaric acid, zinniol, altersolanol, and macrosporin. These toxins cause disease in the host plant by affecting the protoplast of the host and disturb physiological processes that sustain plant health (Agrios, 2005). Respiration decreases and photosynthesis increases partially throughout the production of enzymes and toxins on hosts (Rotem, 1998). *Alternaria solani* may survive for more than ten years in the soil in debris and seeds at optimum temperatures (Rotem, 1998). In controlling leaf disease physical methods are not highly effective under field conditions because they require large investments and difficult to use in large acreages of tomatoes and potatoes (Batista *et al.*, 2006). The two major features of *Alternaria* spp. are the production of melanin in the spores, and the production of non-specific and host-specific toxins of which the earliest identified nonspecific toxins is alternaric acid (Thomma, 2003). This toxin causes chlorosis and necrosis when introduced in tomato plants and also damages non-hosts of *Alternaria* like cabbage, radish, spinach, pea, bean, and others because of its non-specificity (Langsdorf *et al.*, 1990). *Alternaria* has the ability to grow over a wide range of temperatures from 4 to 36 °C and for successful infection it requires a short wet period of at least four hours (Vloutoglou and Kalogerakis, 2000). Best method to control the diseases is through integrated pest management wherein biological control is noteworthy. The general mechanism of biological control can be divided into direct component and indirect effects of the biocontrol agent (BCA) on the plant pathogen. Direct effects include competition for space or nutrients, production of lytic enzyme and antibiotic, inactivation of the pathogen's enzymes and parasitism. Indirect effects include all aspects that produce biochemical and morphological changes in the host plant, such as induced resistance, inorganic nutrients and their solubilization. Plants are capable of producing an immune response after a primary pathogen infection known as systemic acquired resistance (SAR) (van Loon *et al*. 1998). *Trichoderma* spp. are common saprophytic fungi which have received considerable attention due to direct mycoparasitism on pathogenic fungi and effects on plants, such as induced resistance and enhanced plant development (Harman *et al.*, 2004; Yedidia *et al.*, 2000). *Trichoderma* spp. are used in agriculture as biocontrol agents against a number of pathogenic fungi which play an important role in the biological control of soil-borne diseases. In addition, it has been recorded to inhibit the leaf pathogens. Production of antibiotics, competition for nutrients and space and hyperparasitism all play important roles in the antagonistic effect on pathogens by *Trichoderma* (Mukerji and Garg, 1988). With these considerations in mind, studies were conducted to evaluate the biocontrol potential of five *Trichoderma* spp. against *Alternaria solani.*

MATERIALS AND METHODS

Isolation of *Alternaria solani*

Early blight infected tomato leaves were collected from a field located in Federal Urdu University, Karachi and infected leaves were cut into small pieces about 1cm and surface sterilized with sodium hypochlorite (2.0%) for 20 to 30 seconds and again washed with sterile distilled water to remove the disinfectant. The sterilized pieces were plated (4 pieces/dish) in Petri dishes containing potato dextrose agar (PDA) medium, incubated at 26 to 30^{o}C for 1 week. Pure cultures were obtained by sub-culturing.

Isolation of *Trichoderma* spp.

For the isolation of *Trichoderma* spp., 0.005 – 0.015 g soil was sprinkled on the PDA medium in Petri dishes and incubated at 26^{o}C for 1 week. Pure cultures were obtained by sub-culturing and used for the studies. Cultures of some species were obtained from the Department of Agriculture, University of Karachi. In all 5 species of *Trichoderma* were available for evaluation.

Preparation of culture filtrate

Species of *Trichoderma* were grown on potato sucrose broth (PSB) for 12 days at room temperature. The broth was filtered through a sterilized Whatman No 1 filter paper to obtain the culture filtrate. One ml of culture filtrate of a *Trichoderma* sp. was added to 9ml of PSB in a 250ml conical flask with the help of sterilized pipette and a final concentration of 10,000 ppm dilution was obtained. Ten ml from 10,000ppm dilution was transferred into 9ml of PSB in another flask to get a 1000ppm dilution. This process was repeated to get 100, 10 and 1ppm dilutions. Two g agar per 100 ml of PSB was added to each flask and the media were sterilized at 15psi for 15 min and poured in sterilized Petri plates.

An inoculum disc of *A. solani* was introduced in the centre of each Petri plate. The treatments were replicated thrice. The plates were incubated at 28^{o}C and redial growth of the fungus was measured after 24 h interval.

RESULTS AND DISCUSSION

The results of the effects of culture filtrate on growth inhibition of *A. solani* are given in Table 1 while the results of three factor ANOVA are presented in Table 2. All the cultures showed significant inhibitory effect ($p<0.001$). The concentrations were also found to be significant ($p<0.001$). Time (in days) was also highly significant ($p<0.001$). The first and second order interactions were significant (p at the most 0.01). In general, growth of *A. solani* increased in the control as well as in culture filtrates of *Trichoderma* spp., though in the latter the rate of growth was impeded. All five species of *Trichoderma* significantly ($p < 0.001$) inhibited the radial growth of *Alternaria solani.* The growth was suppressed in the order *Trichoderma viride* > *T. harzianum* > *T. pseudokoenigi* > *T. virens* > *T. koenigii*. Radial growth inhibition of *A. solani* increased with increasing concentration of *Trichoderma* filtrates (Table 1).

It has been demonstrated that *Trichoderma* spp. are generally found in all types of soils including orchard and agricultural soils (Roiger *et al*., 1991). Several *Trichoderma* species reduce the infection of soil borne plant pathogens (Sivan and Chet, 1986; Calvet and Berra, 1990; Spiegel and Chet, 1998; Elad, 2000a,b). *Trichoderma harzianum* is an effective biocontrol agent commercially used for soil borne pathogenic fungi (Shalini *et al*., 2006). Siddique *et al*., (2001) reported that five species of *Trichoderma* significantly reduced the nematode population in okra and mungbean. Siddiqui and Shaukat (2004) investigated the effect of *Trichoderma harzianum* on the biocontrol potential of *Pseudomonas fluorescens* against the nematode *Meloidogyne javanica* and concluded that the mixture of *T. harzianum* and *P. fluoresceens* was more effective against the nematode than either biocontrol organism alone. The molecular mechanism of antagonistic activity of *T. harzianum* involves the formation of peptaibol and a hydrolytic enzyme (Schirmbock *et al*., 1994) and possibly 6-penty-α-pyrone metabolite (Scarselletti and Faull, 1994; Cooney *et al*., 1997). *Trichoderma* species have been studied as biological control agents against fungal pathogens (Chet, 1990; Howell, 1998; Siddiqui *et al*., 2001).

Trichoderma is considered as a cellulytic ascomycetes because it is responsible for the destruction of cellulosic fabrics among the organism (Elsas *et al.,* 1997). Lewis and Papavizas (1984) stated that most of the *Trichoderma* species have the ability to aggregate and form chlamydospores in soil or in organic matter after the introduction of the fungus to the soil as conidia. Chet and Baker (1980) stated that acidic pH levels increase the growth of *T. harzianum* and stimulate chlamydospore and conidial germination. Lui and Baker (1980) demonstrated that conidia of *Trichoderma* survive for longer duration in wet compared to dry soil. Chet (1990) reported that the optimal temperature required for growth of *Trichoderma* was around 28°C and growth was very slow below 18°C. Prasun

and Kanthdai (1997) demonstrated that *Trichoderma* overgrew *S. rolfsii* best at 25°C and 30°C in dual cultures. Similarly, Elad and Kapat (1999) and Elad (2000a,b) reported that commercially prepared *T. harzianum* (T39) as Trichodex**R**, has been useful in the control of certain foliar diseases.

Table 1. Mean and SE of fungal growth diameter (cm) of *Alternaria solani* as influenced by different concentrations of *Trichoderma* species.

	Days					
	1	**2**	**3**	**4**	**5**	**6**
Trichoderma viride						
Control	1 ± 0.5	3 ± 0.58	3.93 ± 0.25	6 ± 0.3	7.67 ± 0.28	10 ± 0.5
10000ppm	0.05 ± 0.03	0.1 ± 0.04	0.2 ± 0.06	0.43 ± 0.03	0.47 ± 0.03	0.8 ± 0.06
1000ppm	0.1 ± 0.05	0.160 ± 0.03	0.4 ± 0.05	0.46 ± 0.03	0.86 ± 0.03	0.96 ± 0.03
100ppm	0.15 ± 0.08	0.3 ± 0.05	0.5 ± 0.05	1.5 ± 0.03	1.7 ± 0.05	1.8 ± 0.05
10ppm	0.76 ± 0.03	1.6 ± 0.03	2.4 ± 0.05	2.6 ± 0.05	2.7 ± 0.05	2.8 ± 0.05
1ppm	0.16 ± 0.06	1 ± 0.05	1.4 ± 0.08	1.7 ± 0.05	2± 0.09	3 ± 0.15
T. virens						
Control	1 ± 0	3 ± 0.58	3.93 ± 0.25	6 ± 0.3	7.67 ± 0.28	10 ± 0.5
10000ppm	0.6 ± 0	1 ± 0.09	1.4 ± 0.11	1.8 ±0.08	2.2 ± 0.15	2.5 ± 0.15
1000ppm	0.6 ± 0	1 ± 0.05	1.4 ± 0.11	1.9 ± 0.05	2.23 ± 0.11	2.5 ± 0.05
100ppm	0.4 ± 0	1 ± 0.05	1.4 ± 0.11	1.8 ± 0.05	2.7 ± 0.05	3.13 ± 0.29
10ppm	0.8 ± 0.11	1.6 ± 0.05	2.4 ± 0.05	2.7 ± 0.05	3.7 ± 0.8	4.4 ± 0.11
1ppm	0.2 ± 0.11	1.1 ± 0.05	2.4 ± 0.05	2.7 ± 0.05	3.7 ± 0.05	4.53 ± 0.06
T. harzianum						
Control	1 ± 0	3 ± 0.58	3.93 ± 0.12	6 ± 0.06	7.67 ± 0.28	10 ± 0.5
10000ppm	0 ± 0	0 ± 0	0.05 ± 0.05	0.06 ± 0.05	0.07 ± 0.05	1 ± 0.05
1000ppm	0 ± 0	0.2 ± 0.05	0.4 ± 0.05	0.6 ± 0.05	0.73 ± 0.08	1.36 ± 0.17
100ppm	0 ± 0	0.2 ± 1.96	0.9 ± 0.17	1 ± 0.17	1.3 ± 0.17	1.7 ± 0.11
10ppm	0.8 ± 0.11	1.5 ± 0.11	1.63 ± 0.03	1.8 ± 0.05	2 ± 0.05	2.5 ± 0.08
1ppm	0.2 ± 0.11	1.1 ± 0.05	2.4 ± 0.05	1.7 ± 0.05	2.96 ± 0.26	3 ± 0.15
T. koningii						
Control	1 ± 0	3 ± 0.58	3.93 ± 0.12	6 ± 0.06	7.67 ± 0.28	10 ± 0.5
10000ppm	0 ± 0	2 ± 0.05	2.6 ± 0.05	3 ± 0.12	3.2 ± 0.09	3.5 ± 0.09
1000ppm	0 ± 0	1.8 ± 0.05	1.9 ± 0.07	2 ± 0.07	2.5 ± 0.08	3 ± 0.08
100ppm	0 ± 0	1.8 ± 0.05	1.9 ± 0.05	2 ± 0.05	2. ± 0.05	3 ± 0.07
10ppm	1.1 ± 0.1	2 ± 0.05	2.6 ± 0.05	2.8 ± 0.05	3 ± 0.05	4 ± 0.11
1ppm	1 ± 0	3 ± 0.05	4 ± 0.05	4.4 ± 0.05	5 ± 0.15	5.5 ± 0.28
T. pseudokoningii						
Control	1 ± 0	3 ± 0.58	3.93 ± 0.12	6 ± 0.06	7.67 ± 0.28	10 ± 0.5
10000ppm	0 ± 0	0 ± 0	1.7 ± 0.05	1.8 ± 0	1.86 ± 0.03	2 ± 0.07
1000ppm	0 ± 0	1 ± 0.1	1.4 ± 0.05	1.5 ± 0.05	2 ± 0.05	2.5 ± 0.08
100ppm	0 ± 0	1 ± 0	1.2 ± 0.05	1.4 ± 0.05	2 ± 0.05	2.5 ± 0.12
10ppm	0.8 ± 0.05	1.7 ± 0.05	2.4 ± 0.05	2.46 ± 0.03	3 ± 0.13	3.46 ± 0.24
1ppm	0.2 ± 0.11	1.1 ± 0.11	1.5 ± 0.11	2.8 ± 0.05	3.8 ± 0.05	4 ± 0.11

Table 2. Three factor ANOVA for the results of culture filtrate (factor 1 concentration, factor 2 days, factor 3 culture filtrate).

Source	SS	df	MS	F	P
MAIN EFFECTS					
Concentration	1106.493	5	221.298	12243.983	<0.001
Days	754.517	5	150.9034	8349.1672	<0.001
Culture filtrate	125.496	4	31.33741	1735.864	<0.001
INTERACTIONS					
Concentration × Days	401.343	25	16.0537	888.218	<0.001
Concentration × Culture filtrate	51.308	20	2.56541	141.93903	<0.001
Days × Culture filtrate	29.229	20	1.46149	80.861	<0.001
Concentration × Days × Culture filtrate	24.71	100	0.247105	13.671	<0.001
Error	6.506	360	0.018		
Total	2499.606	539			

$LSD_{0.5}$ (conc) = 0.03941; $LSD_{0.5}$ (Days) = 0.0394; $LSD_{0.5}$ (Culture filtrate) = 0.0359

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