INFLUENCE OF *TRICHODERMA* SPP AGAINST *ALTERNARIA TENUISSIMA* INCITING LEAF SPOT OF *RUMEX ACETOSA* L

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ABSTRACT

Leaf spot of Sorrel (*Rumex acetosa* L.) is caused by *Alternaria tenuissima*. This paper describes the efficacy of *Trichoderma* spp against sensitive and resistant isolates of *A. tenuissima* by dual culture method under *invitro* conditions. *Trichoderma viride T. harzianum*, *T. virens,* T. *koningii* and *T. pseudokoningii* species were used for antagonistic study. Results indicate that all *Trichoderma* species showed antagonistic activity. But among them, *T.viride, T.koningii* and *T.pseudokoningii* showed 80% antagonistic activity than others in case of sensitive isolate of test fungus. Resistant isolate of pathogen was restricting the antagonism in some extent.

Key words: *Rumex acetosa, Alternaria tenuissima, Trichoderma* species dual culture

INTRODUCTION

Vegetables are the most important component of a balanced diet and we can now, grow varieties of different vegetables round the year. India is the world's second largest producer of vegetables next to China. Vegetables are more susceptible to insects pests and diseases due to their tenderness and softness as compared to other crops and virtual absence of resistance characters because of intensive hybrid cultivation (Chiranjeevi *et al*., 2002).The native value of healthy vegetables are altered because of fungal attack and sometimes fungi produce certain mycotoxin in them and make them unsuitable for human consumption.

Garden Sorrel (*Rumex acetosa* L.) is one of the leafy vegetable crop belongs to family polygonaceae. It is an indigenous English plant, common, too in the greater part of Europe, in almost all soils and situations. The medicinal action of Sorrel is refrigerant and diuretic, febrile disorders and in scurvy. Both the root and the seed were formerly esteemed for their astringent properties, and were employed to stem haemorrhage (Cook, 1967).

Therefore, biological control of plant pathogens has been considered as a potential control strategy in recent years and search for these biological agents is increasing. *Trichoderma* is the most commonly used fungal biological control agent and have long been known as effective antagonists against plant pathogenic fungi (Chet *et al*., 1981; Kumar and Mukerji, 1996). During the last decade, species of *Trichoderma* have emerged as the most powerful bioprotectants for the management of a wide variety of plant diseases by virtue of their broad spectrum action against a number of plant diseases caused by fungi, bacteria, viruses and even nematodes (Mukhopadhyay, 2005). Thus the present study was aimed to evaluate the antagonistic activity of *Trichoderma* spp in laboratory conditions.

MATERIALS AND METHODS

Isolation and identification of test pathogen

During Oct 2009 to Jan 2011 surveys was conducted in Sorrel growing areas and markets of Marathwada region of Maharashtra. It suffers severely by leaf spot disease incited by *Alternaria tenuissima* (Fries) Wiltshire*. In vitro* screening with our arbitrary system of bio-antagonists effective against soil borne pathogens is a simplistic approach to understand a small sector of biological system in disease control. Leaf spot infected materials were collected and cut into small pieces (2mm) by sterilized blade. The pieces were then washed with sterilized distilled water thrice and dried by sterilized blotting paper.

In each case, surface disinfested tissue plated on potato dextrose agar (PDA) medium & produced an *Alternaria* species (Simmons, 2007; Subramaniam, 1971;Ellis, 1971).

Isolation of *Trichoderma* spp

Rhizosphere soils of irrigated and non irrigated plants were collected from different parts of Marathwada region of Maharashtra. From the rhizosphere soil samples, desired *Trichoderma* species were isolated by using potato dextrose agar (PDA) and *Trichoderma* selective medium (TSM) by dilution plate technique (Johnson, 1957). The isolated species were identified by reculturing on another petriplates containing sterilized TSM. The isolated species were identified up to species level based on colony characters, growth of fungus and structure of mycelium, conidiophores and conidia (Kubicek and Harman, 2002). All *Trichoderma* spp. were purified by hyphal tip technique. The isolated strains of *Trichoderma* spp were maintained throughout the study by periodical transfers on PDA and TSM slants under aseptic conditions to keep the culture fresh and viable.

Dual culture experiment

The Antagonistic efficacy of different species of *Trichoderma viride*, *T. harzianum*, *T. virens,* T*. koningii* and *T. pseudokoningii* were tested against the isolated sensitive and resistant pathogenic fungus by dual culture experiment (Morton and Stroube, 1955). *Trichoderma* spp. and test fungus was inoculated at 6 cm apart. Three replicates were maintained for each treatment and incubated at 28 ± 2° C for 7 days. Monoculture plates of both served as control. Seven days after incubation (DAI), radial growth of test fungus and *Trichoderma* isolates were measured. Colony diameter of test fungus in dual culture plate was observed and compared with control. The growth inhibition was calculated by using the formula: 100 X C - T / C, Where C = growth in control and T = growth in treatment (Vincent, 1947).

Statistical Analysis

Statistical analyses of the experiments were performed by using the book of Mungikar (1997).

RESULTS AND DISCUSSION

Isolation and identification of test pathogen

Diseased leaf spots were found as a typical reddish spot on leaves, leaf spot variety in size for pinpoint up to 1 to 2 cm in diameter, typically lesions begin as small brown areas that enlarge to about 1 cm in diameter and dark colour spots with concentric rings were appeared. Such symptoms were collected from different locations of Marathwada region of Maharashtra and ten isolates of *Alternaria tenuissim* were isolated. The culture was deposited at CODON Life Sciences Goa and Department of Botany, Arts, Science and Commerce College with ASCNFC-20 and the sequences was submitted to GenBank (Accesssion No.JQ417902).

Isolation of *Trichoderma* spp

Isolates of five species of *Trichoderma*, T*. viride* Pers. *T. harzianum* Rifai, *T. virens* J. Miller, Giddens and Foster, *T. koningii* Oud. and *T. pseudokoningii* Rifai. were isolated from irrigated and non-irrigated rhizosphere soil of Marathwada region of Maharashtra.

Dual culture

Results indicated that all *Trichoderma* species showed antagonistic activity. But among them, *T.viride, T.koningii* and *T.pseudokoningii* showed 80% antagonistic activity than others in case of sensitive isolate of test fungus. Resistant isolate of pathogen was restricting the antagonism in some extent. Overall, all *Trichoderma* species were found more than 50% antagonistic nature (Table1).

Several workers have been reported that the use of *Trichoderma* species against number of plant pathogenic fungi (Brisa *et al.*, 2007; El-Mougy *et al.,* 2007; Harman, 2006).Akbari and Parakhi (2007) reported *T.viride*-I and *T.hamatum*-IV&V isolates showed strong antagonism against *Alternaria alternata* causing blight of sesame. High inhibitory effect of volatile toxic substances emitted by *Trichoderma* spp. on the radial growth of *Fusarium* spp. has also been reported by Dubey *et al*, (2007). The inhibition was high with the direct use of *Trichoderma* spp. in dual culture against *Fusarium oxysporum f sp psidii* (61-69%) & *F.solani* (58-68%) (Gupta and Mishra, 2009). Kumar *et al.* (2007) tested three species of *Trichoderma* i.e. *T.virens, T. viride* & *T. harzianum* against *F.moniliforum var subglutinas* and found them effective. Among the *Trichoderma* species *T. viride* showed the best performance *in vitro* biological control of *Fusarium oxysporum* followed by others(Irfan and Khalid, 2007).

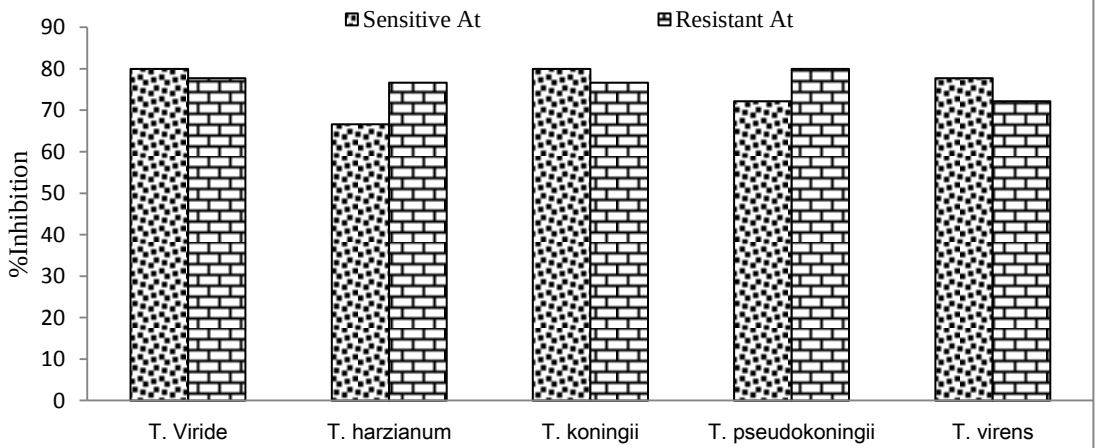
Table 1: Influence of *Trichoderma* species against *A.tenuissima.*

Trichoderma species	Isolates	Radial growth of *A.tenuissima*	Radial growth of *Trichoderma* species	% Inhibition
T. viride	S	20	72	80.00
	R	18	70	77.77
T. harzianum	S	30	60	66.66
	R	21	69	76.66
T. koningii	S	18	72	80.00
	R	21	69	76.66
T. pseudokoningii	S	25	65	72.22
	R	18	72	80.00
T. virens	S	20	70	77.77
	R	25	65	72.22
CD (p=0.05)	3.12			

S=Sensitive, R=Resistant

Trichoderma viride reached the confluence of the Petri dish four days after sowing, so that different fungal isolates occupy a surface of 29% to *Fusarium roseum* (Bouziane *et al*., 2011). Recently, Waghmare and Kurundkar (2011) reported efficacy of *Trichoderma* species against *Fusarium oxysporum* f. sp. *carthami* causing wilt of safflower and isolates no. 29 and 33 were found to minimum growth of the pathogen as compared to others. The species of *Trichoderma* significantly inhibited the mycelial growth of plant pathogenic fungi (Rajkonda *et al*., 2011).

Fig.1: Influence of *Trichoderma* species against *A.tenuissima*



CONCLUSION

Trichoderma species play an important role in controlling fungal plant pathogens. The use of *Trichoderma*-based products is not only safe for the farmers and consumers but it is also good for the environment. However, much more work needs to be done to develop stable, cost effective, easy to produce and easy to apply formulations. Our results concluded that the tested *Trichoderma* spp reduced the growth of pathogen.

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