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Potential deterrence of *Trichoderma* spp. against Fusarial wilt of tomato

S. OJHA AND N. C. CHATTERJEE
Mycology and Plant Pathology Laboratory, Department of Botany, Burdwan University, Burdwan 713 104

Wilt of tomato (*Lycopersicon esculentum*) is the most dreadful disease caused by *Fusarium oxysporum* leading to an enormous economic loss to the growers. An attempt was made to control the disease biologically with the application of antagonists viz. *Trichoderma viride, T. lignorum, T. harzianum, T. hamatum* and *T. reesi*.The effect of volatile and non-volatile antibiotics of *Trichoderma* origin on growth inhibition of the wilt pathogen was studied. *T. harzianum* showed maximum growth inhibition (91%) of the pathogen through mycoparasitism followed by *Trichoderma viride* and the non volatiles produced by these antagonists exhibited their excellent antagonism to the growth of the pathogen (100%) under *in vitro* condition. *Trichoderma* spp. produced siderophore, iron chelating compounds that contributed much towards enhancement of their competitive behaviour for nutrition with the target pathogenic fungi and as such offered their greater antagonistic potentiality. Overall experimental studies clearly indicated *T. harzianum, T. viride* and *T. hamatum* were capable of retarding the growth of the pathogen while *T. lignorum* and *T. reesi* were comparatively less efficient.

Key words : Tomato, wilt, *Fusarium oxysporum, Trichoderma* spp.

INTRODUCTION

Lycopersicon esculentum (tomato) belonging to the family Solanaceae suffering from wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* leads to an enormous loss of tomato cultivation in many countries (Monda, 2002).

The pathogen is soil borne thereby posing a great problem towards strategic management of the disease. Soil borne diseases are difficult to control and seed treatment with fungicides does not protect the crop for long periods. Continuous use of same fungicide for the same pathogen results in the development of fungicide resistant strains of the pathogen and soil application of fungicides in expensive, and also deleterious for associated soil microbiota (Bunker and Mathur, 2001). As an alternative to chemical fungicide in recent times biocontrol agents are gaining importance in the management of the plant pathogens (Manoranjitham *et al.*, 2000).

Fungi have got maximum attention as antagonists probably because of the fact that they are easy to handle and in indentify compared to other microbes (Kundu, 2002). *Trichoderma* isolates are among the most widely researched biological control agents for the protection of agricultural crops from a variety of plant diseases (Papavizas, 1985).

Keeping the above facts in view, the present studies have been undertaken to assess the efficacy and potency of five species of *Trichoderma* in the management of fusarial wilt of tomato.

MATERIALS AND METHODS

The pathogen, *Fusarium oxysporum* was isolated from diseased plants occurring in fields of Burdwan district, West Bengal and maintained in pure line on potato dextrose agar (PDA) slants at 4°C. The identification of the pathogen was confirmed by Indian Agricultural Research Institute, New Delhi (ITCC No. 4123. 2K). The antagonists namely *Trichoderma viride. T. lignorum* and *T. reesi* were isolated from rhizosphere soil of tomato plant and *T. harzianum* and *T. hamatum* were procurred from the Indian Type Culture Collection Centre. IARI, New Delhi.

Antibiosis of the antagonist against the pathogen

In order to study antibiosis against the pathogen, 'food poisoning technique' (Mondal *et al.,* 1995) was adopted.

To obtain the growth metabolites of the antagonists Czapek's synthetic medium was taken as the basal medium. An aliquot of 100 ml of medium was taken in each of the 250 ml flask, plugged and sterilized at 121°C for 15 minutes, cooled and inoculated separately with 5 mm inoculum discs cut out from the margin of actively growing cultures of *T. viride. T. harzianum, T. lignorum, T. hamatum* and *T. reesi.* The flasks were then incubated at 30°±1°C for 15 days. After the incubation period, the mycelial mats from the liquid culture were removed and remainder was filtered through Whatman filter paper No. 1 and passed through a sterilized sintered glass filter (G-5) in order to remove the mycelial bits and spores of the antagonists. The filtrate, thus obtained, served as the growth metabolite of the individual antagonist.

In food poisoning technique 15 ml of sterilized liquid potato dextrose medium at pH 7.4 was poured in the sterilized flask. Soluble metabolites of each antagonist at different doses (1 ml, 2 ml, 5 ml, 10 ml) were mixed separately to the flasks and the flasks were inoculated with inoculum disc of the pathogen (5 mm in diameter) and incubated at 30°±1°C for 7 days. After that mycelial mats were harvested and the percentage of the growth of the pathogen was recorded in Table 1.

Interaction with competition and mycoparasitism by dual culture plating method

'Dual culture plating method' of Royse and Ries (1978) was followed to evalute the competition and mycoparasitism between the pathogen and the antagonists. In this method, 5 mm discs of inoculum of the antagonist and the pathogen were placed simultaneously 3 cm apart on potato dextrose agar plate. The plates were then incubated at 30°C for 7 days. After incubation, the radial growth of both the pathogen and the antagonist was measured and on the basis of growth, inhibition (%) of the pathogen was recorded (Table 2). Petriplate containing only the inoculum of the pathogen served as control. Mycoparasitism was studied following the method of Mumpuni *et al.* (1997) and was measured in terms of 100% colony growth of the pathogen as against the antagonist.

Effect of volatile and non-volatile antibiotics of antagonists on growth of the pathogen

Production of volatile antibiotics was studied using Petridish bases sealed to one another (Dennis and Webster 1971 a,b). An aliquot of 15 ml of PDA medium was poured both in the base and the lid of the petridish. The lid of the petridish was inoculated with 5 mm inoculum disc of the pathogen and the base with 5 mm disc of the antagonist. The base and the lid of the petridish were then fitted with one another and incubated at 30°±1°C .The inhibition (%) of growth of the fungus was recorded (Table 3).

Production of non-volatile antibiotics was estimated by placing a 5 mm inoculum disc of the antagonist centrally on a PDA plate covered by dialyser bag. After 2 days of incubation at 30°±1°C, the antagonist and the dialyser bag were removed. An inoculum disc (5 mm) of the pathogen was then placed centrally on the same PDA plate and incubated at 30°±1°C for 7 days. At the end of incubation, growth rate of the pathogen was recorded (Table 4).

RESULTS AND DISCUSSION

All the five species of *Trichoderma* tested were found to inhibit growth of *F. oxysporum.* Among these five species of *Trichoderma, T. harzianum* and *T. viride showed maximum* efficiency to control the pathogen.

Potential antagonism of *Trichoderma* as evidenced by the results is due to competition, antibiosis and mycoparasitism (Chet *et al.,* 1981; Cook and Banker, 1983; Dutta, 1998; Kundu, 2002; Ozbay *et al.,* 2004; Chakraborty *et al.,* 2005). It was evident from the Table 1 that *Trichoderma harzianum* inhibited the growth of the pathogen with 98.98% efficiency followed by *T. viride* which showed 97.64% inhibition of the pathogen. *Trichoderma* isolates have been reported to produce a wide range of soluble metabolites which are inhibitory to other fungi (Horvath *et al.,* 1995; Dutta, 1998; Chakraborty *et al.,* 2004; Chakraborty, 2005).

Mycoparasitic action of *Trichoderma* has earlier been reported by several workers (Papavizas, 1985; Moon *et al.,* 1995, Mumpuni *et al.,* 1997; Dutta, 1998). In 'dual culture plating' technique *T. harzianum, T. viride* and *T. hamatum* showed 91.11%, 86.67% and 83.33% growth inhibition of *F. oxysporum* respectively. During mycoparasitism *Trichoderma* made intimate contact with the mycelia

Table 1 : Effect of soluble metabolites of the antagonists on growth of *F. oxysporum* following 'food poisoning technique' in liquid medium

Antagonists	*Metabolite of the antagonist (ml.)*	*Dry mycelial weight of the pathogen(mg.)*	*Percentage growth inhibition of the pathogen**
T. viride	1	132.3	10.61±0.16
	2	100.6	32.03±0.995
	4	73.72	50.19±1.79
	5	42.4	71.35±3.95
	10	3.5	97.64±1.34
T. harzianum	1	124.5	15.88±1.79
	2	92.3	37.64±2.38
	4	53	64.19±1.94
	5	28.9	80.47±2.01
	10	1.5	98.98±3.48
T. lignorum	1	140.6	5±0.71
	2	118.7	19.8±1.41
	4	95.8	35.27±1.72
	5	54.2	63.38±0.76
	10	18.3	87.64±2.71
T. hamatum	1	135.7	8.31±0.99
	2	112.5	23.99±0.9
	4	84.9	42.64±0.75
	5	46	68.92±0.93
	10	12.8	91.38±0.67
T. reesi	1	141.9	4.12±0.70
	2	127.2	14.05±0.55
	4	102.4	30.81±0.39
	5	62.5	57.77±0.49
	10	28.7	80.61±0.73
Control	0	148	0

SEM = ±6.24 ; CD at 5% = 13.291
* Data are the mean values of five replicates

Table 2 : Antagonistic potention of *Trichoderma* sp. through mycoparasitism on growth of *F. oxysporum* following 'dual culture plating method'

Antagonists	*Radial growth of the pathogen (cm.)*	*Radial growth of the antagonist (cm.)*	*Growth inhibition of the pathogen* (%)*
T. viride	1.2	7.8	86.67±0.93
T. harzianum	0.8	8.2	91.11±0.35
T. lignorum	2.1	6.9	77.27±1.23
T. hamatum	1.5	7.5	83.33±0.78
T. reesi	2.22	6.78	75.51±0.93
Control	9	0	0

SEM = ±1.24 ; CD at 5% = 2.641
* Data are the mean values of five replicates

of target fungi, grew over and coiled around the hyphae of the host fungi and penetrated the host tissues (Banker and Dickeman, 1993; Kumar and Gupta, 1999). *Trichoderma* antagonists have been reported to solubilize the host mycelia by the action of different hydrolytic enzymes they produce and finally feed on the host hyphal contents (Aziz *et al.*, 1993; Shwet *et al.*, 2000; Chakraborty *et al.*, 2004).

Table 3 : Antagonistic effect of volatile antibiotics produced by *Trichoderma* spp. on growth of *F. oxysporum*

Antagonists	*Radial growth of the pathogen(cm.)*	*Growth inhibition of the pathogen(cm.)*	*Growth inhibition of the pathogen*(%)*
T. viride	2.33	6.66	73.99±0.75
T. harzianum	1.9	7.1	78.89±0.78
T. lignorum	3.22	5.78	64.22±0.74
T. hamatum	2.8	6.2	68.89±0.78
T. reesi	3.68	5.32	59.11±0.82
Control	9	0	0

SEM = ±1.46 ; CD at 5% = 3.110
* Data are the mean values of five replicates

Table 4 : Antagonistic effect of non-volatile antibiotics produced by *Trichoderma* spp. on growth of *F. oxysporum*

Antagonists	*Radial growth of the pathogen(cm.)*	*Growth inhibition of the pathogen(cm.)*	*Growth inhibition of the pathogen*(%)*
T. viride	0	9	100±0
T. harzianum	0	9	100±0
T. lignorum	0.36	8.64	96.00±0.57
T. hamatum	0.2	8.8	97.78±0.35
T. reesi	0.5	8.5	94.44±0.35
Control	9	0	0

SEM = ±0.47 ; CD at 5% = 1.001
* Data are the mean values of five replicates

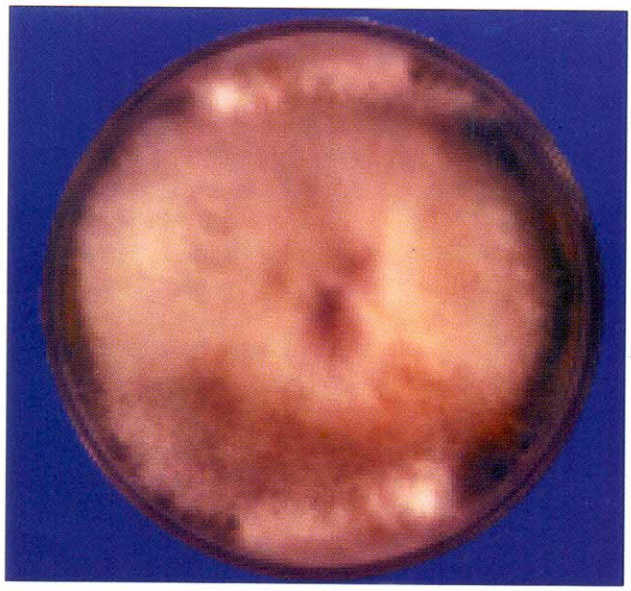

Fig. 1 : Fully grown culture of *F. oxysporum*.

The volatile and non volatile antibiotics produced by *Trichoderma* spp. are inhibitory to soil borne plant pathogens (Jackson *et al.*, 1991; Cliquet and Scheffer, 1996; Burce *et al.*, 2000) including *F. oxysporum* (Mukhopadhyay and Kaur, 1990;

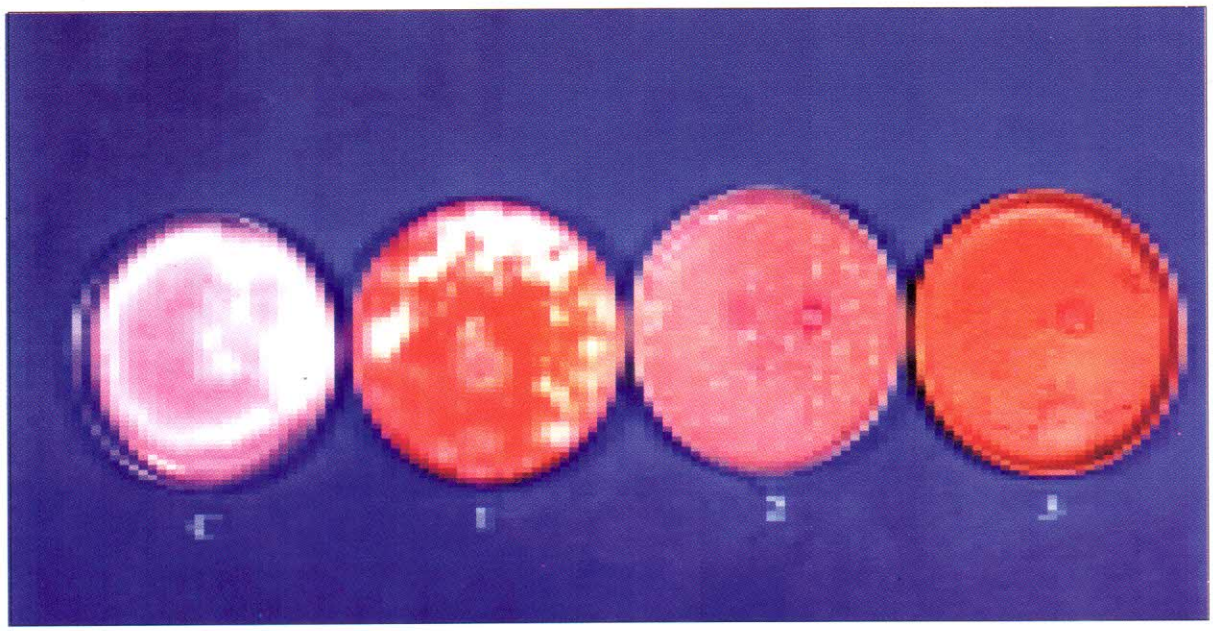

Fig. 2 : Showing the effect of growth metabolites of *T. harzianum*, gradual growth inhibition of *F. oxysporum occurs with the increase of doses of metabolites where C = control plate.*

Monda, 2002). It is evident that all the five *Trichoderma* spp. were capable of producing both volatile and non volatile antibiotics in culture (Table 3 and Table 4) and through the production of these two categories of antibiotics *Trichoderma* spp. possessed a strong inhibitory action towards the test fungi. Non volatiles produced by the antagonists were found to arrest the growth of the test fungi completely. Volatile antibiotics produced by *Trichoderma harzianum* showed 78.89% growth inhibition of pathogen follwed by *T. viride* (74.11%). Diffusible (non-volatile) antibiotic activity of *Trichoderma* spp. that happened to be more effective than the volatile antibiotics (Bunker and Mathur, 2001) corresponds with the present findings. Some antibiotics produced by *Trichoderma* are suzukacillin (Ooka *et al.,*1966), viridin (Webster and Lomas, 1964), dermadin (Pyke and Dietz, 1966) etc. which may be fungistatic or fungicidic in nature (Jackson *et al.,* 1991; Mondal *et al.,* 1995). *Trichoderma* spp. showed interspecies variability in their antagonistic potentiality due to presence of variable amount of antifungal metabolites, ability to produce lytic enzymes and iron chelating compounds, siderophores. From the above experiments *Trichoderma harzianum* is proved to be the most efficient biocontrol agent that shows greater antagonistic property towards the control of wilt pathogen, *F. oxyporum.*

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