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Aspergillus niger, a potential biocontrol agent for controlling fusarial wilt of tomato

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Wilt disease caused by *Fusarium oxysporum* f.sp. *lycopersici*, (FOL) is considered to be the most important disease of tomato. The present study was carried out to find a potential biocontrol agent for the management of FOL. Different kind of bioagents like *Trichoderma* spp. (28 isolates), *Aspergillus niger* (one isolate), *Pseudomonas* spp. (10 isolates) and *Bacillus* spp. (13 isolates) were isolated from the rhizosphere of the crop and evaluated for their *in vitro* efficacy against FOL. Among them *A. niger* recorded 74.0 % inhibition of mycelial growth of FOL followed by *T. viride*-Tv5 (62.4%), *T. harzianum*-Th4 (61.1%), *B. subtilis*-Bs-2 (31.2%) and *P. fluorescence*-P-3 (27.0%). Similarly, all the biocontrol agents were tested against the wilt incidence under glasshouse conditions. Among the treatments, *A. niger* recorded 0% wilt incidence on 30 and 45 days after inoculation, where as, the protection shown by *T. viride* (Tv5), *T. harzianum* (Th4) and *P. fluorescens* (P3) on 30 days after inoculation was broken down on 45th day after inoculation. From this study *A. niger* identified as potential biocontrol agent of FOL and further investigations on development of bioformulation and biosafety are in progress.

Key words : *Aspergillus niger*, biocontrol, *Fusarium*, tomato.

INTRODUCTION

Fusarial wilt of tomato (*Solanum lycopersicum*) caused by *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) Snyder and Hansen is one of the devasting soil borne diseases of tomato. The initial symptoms of this disease are yellowing of foliage started from lower leaves and later stage, development of browning in vascular regions (xylem). The vascular wilt disease is most destructive both in greenhouse and field conditions and causes 10% to 80% yield losses (Mao *et al*, 1998; Banerjee *et al.*, 1990). Constant use of chemical fungicides for the control of this disease is common practice but the harmful effect of these chemicals limit scope of the routine application. Alternatively, management of this disease through use of biocontrol agents is trustworthy. Biocontrol agents of both fungal (Khan and Khan, 2001; Singh *et al.*, 2002; Shahnaz Dawar *et al.*, 2008; Padmodaya and Reddy, 1996; Srinon *et al.*, 2006) and bacterial (Benehabane *et al.*, 2000; Fakhouri and Buchenauer, 2003; Ghonim, 1999; Khan and Khan, 2001; Guo *et al*, 2004) have been registered against pathogens of soil borne nature. The main aim of this study is to isolate and select the antagonistic fungi and bacteria which are effective against *F. oxysporum* f.sp. *lycopersici* (FOL).

MATERIALS AND METHODS

Isolation and purification of pathogen and bio control agents

Tomato plants showing common symptoms of vascular browning were collected from different agroclimatic regions of India and isolated on potato dextrose agar medium (PDA) using standard procedure. Different vegetable rhizosphere soil samples were collected from Indian Institute

of Vegetable Research, Varanasi Antagonistic fungi and bacteria were isolated using dilution plate technique on PDA and nutrient agar (NA) media respectively. Different antagonistic microbes were identified based on morphological characters.

Pathogenicity test

All the collected isolates of FOL were tested for pathogenicity on tomato (cv. Kajala) by root cut and dip method to find out their virulence under greenhouse conditions. The seedlings roots were dipped in the FOL conidial suspensions (1×10^6 spores/ml) for 30 min then transplanted into the pots containing sterilized soils. After transplanting the pot soils were drenched with the spore suspension (@ 20 ml/pot). After 45 days observations were taken on disease development to find out the virulence level (Krnjaja *et al*, 2005).

In vitro experiments

Efficacy of different biocontrol agents against the virulent isolate of FOL were evaluated by dual culture experiment. Inhibition of mycelial growth of FOL colony in the presence of antagonistic microorganism was measured and per cent inhibition was calculated (Vincent 1947), with the following formula :

I = C-T/C x 100, where I = Per cent inhibition of the mycelial growth, C = mycelial growth in control, T = mycelial growth in treatment.

Greenhouse experiments

The effective biocontrol agents were evaluated for their efficacy against wilt disease under greenhouse conditions. Pots containing sterilized soil mixture (soil, sand and FYM in 2:1:1 ratio) were inoculated with FOL spore suspensions as stated in pathogenicity test. Talc based formulation of biocontrol agents were prepared as per the standard protocol (Vidhyasekaran and Muthamilan 1995.) and applied in pots @ 10 g/kg of soils. Seedlings of "Kajala" (20 days old) were transplanted after dipping in talc based formulation of biocontrol agents (10 % solution) for 30 min. Per cent Disease Intensity (PDI) was assessed 15, 30 and 45 days after transplanting (Banerjee *et al.*, 1990; De Cal *et al.*, 1999), and calculated according to the formula : PDI = Sum of the numerical grade/Maximum category number x No. of plants observed x 100

RESULTS AND DISCUSSION

Evaluation of cultivar for resistance and studying the virulence nature of pathogenic isolates needs pathogenicity test (Krnjaja *et al.*, 2005). Sum of 24 isolates were collected from different parts of India, isolated and purified (Table 1). Based on the pathogenicity of the isolates were grouped into different categories more interestingly all the isolates which were collected from Uttar Pradesh and one isolate each from Assam, Tamil Nadu and Sikkim were severely virulent (Table 1). For further experiments *viz.*, dual culture and green house studies a strong virulent isolate FOL-VA-1 was used. Among 52 biocontrol agents used, *A. niger* recorded the highest inhibition (73.98 %) against the strong virulent isolate of FOL-VA-1, followed by *T. viride* Tv5 (62.43%) and *T. harzianum* Th4 (61.12%) (Table 2). The inhibition effect of tested bioagents is possibly due to the production of cell wall degrading enzymes, antibiotics and competition (Lawton and Lamb, 1987; Schneider and Ullrich, 1994; Dalisay and Kuc, 1995; Xue *et al.*, 1998; Sharma and Dureja 2004). This effectiveness may vary due to the nature, quality and quantity of the inhibitory substance(s) secreted by bioagents (Skidmore and Dickinson, 1976).

In greenhouse experiment, up to 15 days after inoculation (DAI) there was no wilt development on cv. "Kajala' which indicated that more than 15 days are required for the establishment of wilt disease (Table 3). After 30th day of inoculation, observations were made on initial symptoms shown at different treatments. Among the treatments, *A. niger* recorded 0% wilt incidence followed by *T. viride* Tv5 (6.66%), *T. harzianum* Th4 (13.33%) and *P. fluorescens* P3 (13.33%). More importantly, at 45th DAI, more than 90% symptoms were observed on control and there was 0% incidence noticed in *A. niger* treatment, where as, the protection shown by *T. viride* Tv5, *T. harzianum* Th4 and *P. fluorescens* P3 on 30 days after inoculation was broken down on 45th day after inoculation. Though already popularized biocontrol agents *viz.*, *Trichoderma*, *Pseudomonas* and *Bacillus* were failed to com-

Table 1 : Effect of different FOL isolates on pathogenicity reaction on tomato (cv kajala)

Name of the FOL isolates	Plance of collection	Grade of pathogenicity	Reaction
FOL-VA-1	Araziline, Varanasi, UP	3.0	Strong virulence
FOL-VA-2	Khanav, Varanasi, UP	2.9	Strong virulence
FOL-VA-3	Kelabela, Varanasi, UP	2.8	Strong virulence
FOL-VA-4	Kaneri, Varanasi, UP	2.9	Strong virulence
FOL-JA-1	JNKVV Farm, MP	1.6	Medium virulence
FOL-RA-1	IGKVV Raipur, CG	1.6	Medium virulence
FOL-BI-1	Sindhari Kachhar, Bilaspur, CG	1.3	Medium virulence
FOL-HY-1	Rajendranagar, Hyderabad, AP	1.3	Medium virulence
FOL-HY-2	APAU Farm, Hyderabad,	1.3	Medium virulence
FOL-NA-1	Kalyani, Nadia, WB	1.3	Medium virulence
FOL-PA-1	24 Pargana, WB	0.8	Weak virulence
FOL-LU-1	PAU Ludhiana, Punjab	1.6	Medium virulence
FOL-JO-1	AAU Jorhat, Assam	1.6	Medium virulence
FOL-AS-1	KVK Guwahati, Assam	2.3	Strong virulence
FOL-BH-1	OUAT Bhubaneshwer, Orrisa	0.8	Weak virulence
FOL-BH-2	Bhubaneshwer, Orrisa	0.8	Weak virulence
FOL-JP-1	Jaipur, Rajasthan	1.6	Medium virulence
FOL-CO-1	Coimbatore, TN	1.6	Medium virulence
FOL-CO-2	Coimbatore, TN	2.8	Strong virulence
FOL-TP-1	Lankamura, Tripura	1.6	Medium virulence
FOL-TP-2	Samura, Agartala, Tripura	1.6	Medium virulence
FOL-SK-1	Asamlengey, Sikkim	2.3	Strong virulence
FOL-MG-1	Nampoh, Shillong, Meghalaya	0.3	Weak virulence
FOL-GU-1	Junaghar, Gujrat	1.6	Medium virulence

Table 2 : *In vitro* inhibition of mycelial growth of FOL by different biocontrol agents

Treatment	Growth of FOL isolates on 9th day (mm)	% Inhibition (9th day)*
A niger	20.3	73.98^{c}
T. viride	29.3	62.43^{d}
T. harzianum	30.3	61.12^{d}
B. subtilis	53.7	31.21^{c}
P. fluorescens	57.0	26.92^{d}
Control	78.0	00.00^{a}

*Values were are sine transformed before the analysis.

Table 3 : Effect different biocontrol agents on wilt disease incidence in tomato under greenhouse conditions.

Treatment	PDI		
	15 DAI	30 DAI	45 DAI
A niger	0.00 ns	0.00^{a}	00.00^{a}
T. viride	0.00 ns	6.66^{b}	40.00^{a}
T. harzianum	0.00 ns	13.33^{c}	26.67^{b}
B. subtilis	0.00 ns	13.33^{c}	53.33^{c}
P. fluorescens	0.00 ns	20.00^{d}	33.33^{c}
Control	0.00 ns	46.67^{c}	93.33^{1}

*Values were are sine transformed before the analysis. In a column a mean followed by common letters are not significantly different at 5% level by DMRT.

bat the FOL wilt disease in tomato. But *A. niger* could protect upto 45 DAI which indicated that the biocontrol identified in this study could be further utilized for formulation development and field application.

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