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Short communication

Biocontrol efficacy of fungal and bacterial antagonists against early blight of tomato caused by *Alternaria solani*

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ABSTRACT

In the present study, fungal and bacterial antagonists were evaluated against early blight pathogen of tomato, *Alternaria solani* under *in vitro*, glasshouse and field conditions. Among the isolates tested, *T. harzianum* (Th-7) showed significant inhibition of *A. solani* under *in vitro* (72.78%) and glasshouse conditions (62.60%). Seedling dip and foliar applications of *T. harzianum* (Th-7), *T. viride* (Tv-14) and *P. fluorescens* (Pf-1) decreased the early blight incidence up to 62% and increased tomato yield up to 37% over control in field trials.

Key words: Tomato, *Alternaria solani*, biocontrol, *Trichoderma* sp., *Pseudomonas fluorescens*.

Tomato (*Solanum lycopersicum* L.) is susceptible to variety of fungal diseases, among which, early blight of tomato caused by *Alternaria solani* is a serious problem (Gleason and Edmonds, 1). At present, the management of this disease is mainly dependent on application of chemical fungicides, which has several disadvantages like toxic residues, elimination of beneficial micro-flora, development of resistance in pathogens to fungicides and environment pollution. Hence, eco-friendly biocontrol strategies have to be developed for managing this disease (Heydari and Pessarakli, 2). Most of the studies carried out on biological control of *A. solani* were confined to laboratory/ glasshouse conditions. At present, there is a need to identify the highly efficient strains of antagonists for management of *A. solani* in tomato under field conditions and, hence, the current investigations were conducted.

Forty eight isolates of different *Trichoderma* species (*T. viride* Tv-1 to Tv-16; *T. harzianum* Th-1 to Th-21; *T. virens* Tvs-1 to Tvs-8; *T. hamatum* Tham-1; *T. koningii* Tk-1 and *T. pseudokoningii* Tpk-1), one isolate of *Pseudomonas fluorescens* (Pf-1) and *Bacillus subtilis* (Bs-1) were obtained from NBAII culture collection for screening against *A. solani*. *In vitro* effect of fungal and bacterial antagonists against *A. solani* was tested by dual culture test (Webber and Hedger, 5). Pot culture experiment was conducted to evaluate the efficacy of 19 promising isolates of *Trichoderma* species (*T. viride* Tv-4,7,9,10,13,14; *T. harzianum* Th-2,4,7,8,9,16,18; *T. virens* Tvs-1,2,3,4,5; *T. pseudokoningii* Tpk-1), one isolate of *P. fluorescens* and *B. subtilis* in randomized design with five replications (5 seedlings/replication). Tomato seedlings (Hybrid 1389, Syngenta) were raised in earthen pots (20 cm × 18 cm) filled with potting mixture pre-sterilized in an autoclave at 121°C for 60 min. Foliar application of antagonist suspensions (*Trichoderma*: 2×10^6 cfu/ml; Bacterial: 1×10^8 cfu/ml) and *A. solani* (2×10^4 spores/ml) was given simultaneously to tomato seedlings. Mancozeb (3.0 g/l) served as fungicidal check and plants sprayed with *A. solani* alone served as control. Observations on development of early blight symptoms were recorded after ten days of inoculation. Disease severity was assessed by following 0-9 scale according to Ramakrishnan *et al*. (4) and percent disease index (PDI) was estimated using McKinney (3) formula. The glass house study was conducted twice and the pooled data is presented. Field trials were conducted in *kharif* season for two years in randomized block design with individual plot size of 5 m × 2 m replicated thrice (25 plants/ replication). Biocontrol efficacy of twelve promising isolates of *Trichoderma* sp. (*T. viride* Tv-4, 10, 13, 14; *T. harzianum* Th-7, 9, 16, 18; *T. virens* Tvs-2, 3, 4, 5) and *P. fluorescens* and *B. subtilis* were evaluated under field conditions. Invert oil formulations of *Trichoderma* sp. (2×10^6 cfu/ml) and talc formulations of bacterial isolates (1×10^8 cfu/g) were applied as seedling dip (10 min.) during transplantation (10% in 1l of water) and foliar sprays (10% in 1l of water) at monthly intervals up to 4 months. Mancozeb (3 g/l) served as fungicidal check and unsprayed plants served as control. Disease severity was assessed and the pooled data from two years are presented. The data obtained from all experiments were analyzed in completely randomized block design after arcsine transformation with analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) was used to

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Table 1. Efficacy of *Trichoderma* and bacterial antagonists against *Alternaria solani*.

Treatment	*In vitro* inhibition (%)	Glasshouse studies		Field conditions		Yield (tonne/ha)
		Disease severity (%)	Reduction over control (%)	Disease severity (%)	Reduction over control (%)	
Tv-7	63.66[b]	23.28[b]	51.70	48.46[d]	8.79	48.72[d]
Tv-10	60.36[c]	23.40[b]	51.45	27.63[b]	48.00	53.33[b]
Tv-13	60.89[b]	23.88[b]	50.45	40.06[c]	24.61	55.00[c]
Tv-14	61.42[b]	22.70[b]	53.86	20.33[a]	61.74	59.92[a]
Th-7	72.78[a]	18.40[a]	62.60	20.66[a]	61.12	58.12[a]
Th-9	65.80[b]	20.43[b]	56.76	22.16[a]	58.29	58.83[a]
Th-16	61.26[b]	22.40[b]	52.59	52.6[d]	1.00	46.28[d]
Th-18	63.72[b]	21.05[b]	55.44	52.0[d]	2.13	46.67[d]
Tvs-2	58.95[c]	24.01[b]	50.18	45.13[c]	15.06	52.12[c]
Tvs-3	58.35[c]	22.66[b]	53.11	42.93[c]	19.02	54.77[c]
Tvs-4	52.55[d]	22.94[b]	52.40	30.56[b]	42.49	55.68[b]
Tvs-5	53.95[d]	22.50[b]	53.31	45.76[c]	13.88	51.85[c]
Pf-1	52.32[d]	22.42[b]	51.08	21.36[a]	59.79	59.25[a]
Bs-1	51.23[d]	22.58[b]	50.73	28.50[b]	46.35	55.67[b]
Fungicide	-	12.67[a]	73.18	17.26[a]	67.51	60.53[a]
Control	0.00[e]	47.25[c]	-	53.13[d]	-	43.87[d]

Mean values followed by the same letters within each column are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$)

compare means. Statistical analyses were performed using AgRes statistical software, version 3.0 for Windows.

In the *in vitro* studies, *T. harzianum* (Th-7) showed significantly higher inhibition (72.78%) of *A. solani* (Table 1). In glasshouse studies, plants treated with *T. harzianum* (Th-7) showed significantly higher disease reduction (62.60%) over untreated control. Other *Trichoderma* isolates and bacterial antagonists, *P. fluorescens* and *B. subtilis* showed disease reduction 50.18 to 56.76% and were statistically on par with each other (Table 1). In the field trials, seedling dip and foliar applications of *T. viride* (Tv-14) and *T. harzianum* (Th-7 and Th-9) and *P. fluorescens* (Pf-1) showed less disease severity (20.33 to 22.16%) compared to untreated control (53.13%). The treatment with these antagonists decreased the disease severity up to 61.74% and increased tomato fruit yield up to 36.58% over control (Table 1). The stepwise screening followed in the present study has helped in identifying the potential antagonist like *T. harzianum* (Th-7), which has suppressed *A. solani* under laboratory, glasshouse and also in field conditions. The present study gives a comparison among widely used biocontrol agents like different *Trichoderma* spp. *P. fluorescens* and *B. subtilis* with respect to their biocontrol efficacy against early blight disease. The study has helped in identifying potential biocontrol agents, *T. harzianum* (Th-7 and 9), *T. viride* (Tv-14) and *P. fluorescens* (Pf-1) for management of early blight of tomato under field conditions.

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