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Inhibition of Wilt Disease by Antagonist and its Effect on Shoot Length, Root Length and Biomass of Tomato Plant

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Keywords: *Fusariumoxysporum f.sp. lycopersici, antagonists (Tricordermasps, Bacillus subtilis) root & shoot length,biomass, tomato plant.*

INTRODUCTION

*Fusarium*wilt disease is one of important disease of tomato and is very destructive whenever tomatoes are grown intensively as reported by Butler (3). Every known commercial variety has been attacked by the wilt disease which causes great loss in the commercial cultivation of tomato. In order to eliminate all the impediments to maximum production of food it is imperative to control pathogen by various measures, Singh (22); Raj & Kapoor (20). Besides the use of various chemical methods, use of antagonists to suppress the pathogen are also reported by Gaikwad, *et.al.* (8) .*Trichoderma* spp. are recognized asa potential biocontrol agents against several soil borne diseases. The aim of the present work is to study the effect of several biocontrol agents on the *Fusarium* wilt disease and in the growth of tomato plant.

MATERIASLS AND METHODS

Fusarium wilt disease was found high in these plots during survey. Locality 1 = (Soil A) Collected from village Ghoroniadohmile, Dibrugarh Assam. Locality 2 = (Soil B) Collected from village Phukanbam, Dibrugarh, Assam.

Soil Inoculation

The test fungus (*Fusariumoxysporum*f. sp. *lycopersici*) was multiplied in sand maize meal medium (dry sieved sand – 90g, maize meal – 10 g, Distilled water – 40 ml) and asepticallyincubated at 27±1°C for 3 week after sterilized. Mass culture of *F.o.*f. sp. *lycopersici* was added to 5 kg sterilized soil at 10% w/w basis and allowed for stabilization for a week, Sen and Kapoor, (26). Earthen pot of 10 cm face diameter was used throughout the experiment. *Trichoderma* species (*T. viride, T. harzianum* and *T.koningi*) were multiplied in wheat bran sand medium, tap water – 500ml, at the ration of 1:1:2 w/w/v for 1 week at 26 ± 2°C following method ofGangadharan & Jeyarajan, (9) and inoculated in each pot at 2% (w/w) of soil containing *Fusarium* infested soil. Bacterial antagonist *B. subtilis* was multiplied in nutrient broth (peptone – 10g, Lemco – 10g, NaCl – 0.59g, Distilled water – 1 Litre, Peptone and Lemco boiled in small amount of distilled water. Then the solution is filtered and pH is adjusted to 7.5 make 1 Litre and added to the *Fusarium* infested soil at 20ml/pot. 15 plants were tested against each treatment. Control pot are maintained with *F.o.* f. sp. *lycopersici*. Data on wilt disease incidence were recorded up to 8week,andanalysed statistically.

Root Dip Treatment:-Mass culture of *Trichoderma sp* on PDA for 1 week were prepared and flooded with sterile distilled water to prepare spore suspension containing 3-6 $\times 10^6$conidia / ml . Bacterial antagonist *B. subtilis* was taken and inoculated in each pot at 2% w/w of soil containing *Fusarium* infested soil. Tomato seedling (Cv. Pusa Ruby) of four week old seedlings were removed, roots were thoroughly washed and immersed in spore/bacterial

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cell suspension of antagonists for 30 minutes. Three seedlings were transplanted in each pot containing *Fusarium* infested soil. Disease incidence was recorded using a 0 to 5 scale to cover each symptom criterion. Mean disease score (MDS) and percentage disease incidence (PDI) were calculated by

$$\text{MSD} = \frac{\text{Sum total of score}}{\text{Total number of plant assessed}}$$

$$\text{PDI} = \frac{\text{MDS}}{\text{Maximum Grade}} \times 100$$

Observation for disease incidence (DI) were recorded from appearance of disease (3^{rd} week) upto 8 week after planting (WAP). Percentage disease reduction was calculated by using formula :

$$\text{PDR} = \frac{\text{DI in inoculated check} - \text{DI in treatment}}{\text{DI in inoculated check}} \times 100$$

For reduction of disease growth of the host plant (root and shoot length, biomass) were also done after treatment of antagonist. Percentage reduction in mean shoot length and mean root length (MRL) of tomato plant was calculated by using formula—

% Reduction in shoot and root length

= MSL/MRL (Healthy) – MSL/MRL

(Treatment × 100 MSL/MRL (Healthy)

Biomass of Tomato Plants

Tomato plant were harvested carefully along with their roots at the end of the growing season. Plant parts were oven dried at 60 ± 2°C for 24 to 48 hour and weighed separately and expressed in fresh weight and dry weight, Hickman (14).

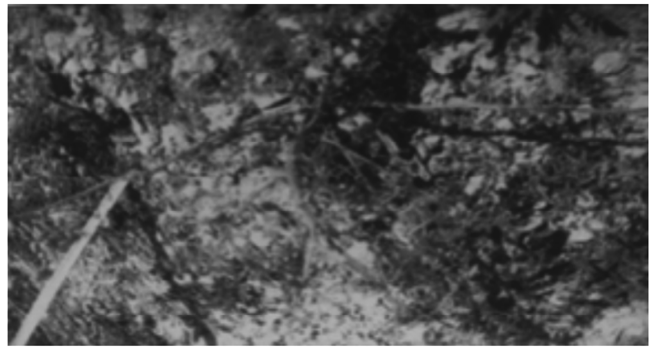

Figure 1: ***Fusarium*** **wilt in tomato**

RESULT & DISCUSSION

Application of antagonists *T. viride* and *B. subtilis* in Soil A through root dip treatment and through soil inoculation (Table 1) showed disease reduction upto 70% & 57.50% and 67.50%, & 50% respectively. In *T. harzianum* and *T. koningi* disease inhibition recorded 37.50% & 32.50% and 10% & 7.5% through both root dip and soil inoculation method. Application of antagonists *T. viride* and *B. subtilis* in Soil B through root dip method also showed disease reduction upto 68.57% & 57.14% (Table 1). While in soil inoculation reduction of disease was found 63.42% and 52% by application of *T. viride* and *B. subtilis*.

Application of *Trichoderma* spp, *Bacillus subtilis, Pseudomonas* sp. are reported to reduce disease incidence in several crops including tomato (Elad *et. al.* (7).; Goricagomez, (10), Podile & Dube, (18),Padmodaya and Reddy (19). Hadar *et. al.* (13) has been reported that *Rhizoctonia* damping off disease of bean, tomato, egg plant could be controlled by the application of *T. harzianum* in the glass house. Rasal & Patil (21) also reported the growth of

Table 1
Effect of Antagonist on wilt disease of tomato caused by *F.o.f.sp.lycopersici*.

Treatment	*Soil A (Root Dip)*			*Soil A (soil inoculation)*			*Soil B (Root Dip)*			*Soil B (Soil inoculation)*		
	8 WAP	*PDI*	*%DR at 8WAP*	*8WAP*	*PDI*	*%DR at 8WAP*	*8WAP*	*PDI*	*% DR 8WAP*	*8WAP*	*PDI*	*%DR at 8WAP*
Fo + Tv	1.2	24	70	1.3	26	67.5	1.1	22	68.57	1.28	25.6	63.42
Fo + Th	2.5	50	37.5	2.7	54	32.5	2.33	46.66	33.34	2.42	48.4	30.55
Fo + Tk	3.6	72	10	3.7	74	7.5	3.3	66	5.71	3.4	68	2.85
Fo + Bs	1.7	34	57.5	2	40	50	1.5	30	57.14	1.7	33.6	52
Control (Fo)	4	80		4	80		3.5	70		3.5	57.14	
Healthy												
SEd (±)	0.230			0.086			0.311			0.309		
CD (P = 0.05)	0.479			0.180			0.648			0.673		
CD(P = 0.01)	0.634			0.244			0.950			0.943		

Fo = *F.o.*f. sp. *Lycopersici Th* = *T. harzianum Tv* = *T. viride* Tk = *T. koningi Bs* = *B.subtilis (–)* = *Nil*
DR-Disease Reduction

Table 2
Effect of Antagonist on the growth of tomato plant in Soil A (root dip & soil inoculation).

Treatment	*(MSL) (cm)*	*(MRL) (cm)*	*R% SL*	*R% RL*	*(MSL) (cm)*	*(MRL) (cm)*	*R% SL*	*R% SL*
	Root Dip				*Soil inoculation*			
Fo + Tv	46	11	11.64	8.25	45.93	11.34	11.77	5.42
Fo + Th	38.90	10	25.27	16.59	39.86	10	23.43	16.59
Fo + Tk	21	5.8	59.66	51.62	21.33	5.80	59.02	51.62
Fo + Bs	44.60	8.94	14.32	25.43	44.33	9.92	14.84	17.26
Control (C_1) (inoculated)	21.13	5.34	59.41	55.46	21.13	5.34	59.41	55.46
Control (C_2) (Uninoculated)	52.06	11.99			52.06	11.99		
SEd (±)	0.967	0.304			0.544	0.370		
CD (P = 0.05)	1.934	0.608			1.088	0.740		
CD (P = 0.01)	2.751	0.864			1.547	1.052		

Data are mean of 15 plants/treatment
C1 = *only F.of.sp.lycopersici*
Fo = *Fusarium oxysporum* f. sp. *Lycopersici*
C2 = without *Tv.Th.Tk.Bs*
Th = *Trichoderma harzianum*
Tv = *T. viride*
Tk = *T. koningi*
Bs = *Bacillus subtilis*

*Fusariumoxysporum*f. *vasinfectum*and reduction in the mortality of cotton plant by application of *T. viride*. The parasitic activity of *T. harzianum* against various members of soil borne plant pathogens was reported by Mehta *et. al.* (15). Application of *Trichoderma* spp and *Bacillus subtilis* through seed treatment and seedling root dip as well as soil application are reported to reduce disease incidence in several crops as reported by Mukhopadhyay *et. al.*, (16); Saikia *et. al.* (24); Mean shoot length (MSL) and Mean root length (MRL) of tomato plant after treatment of antagonist in soil A through root dip treatment was found maximum *i.e.* 52.06 cm & 11.99 cm in healthy plant and minimum *i.e.* 21.13 & 5.34cm in control where there was no antagonists. Application of antagonist *T. viride* showed increased MSL & MRL of tomato plant 46cm & 11cm as compared to control. In *B. subtilis* MSL & MRL of tomato plant was found 44.60cm & 8.94cm. While in *T.harzianum* it was recorded 38.90cm & 10cm. In *T. koningi* no significant increase in MSL & MRL was found .(Table-b & c). MSL & MRL of tomato plant after application of antagonists in Soil A through soil inoculation are found effective *i.e.* 45.93cm & 11.34cm, 44.33cm & 9.92cm, respectively. MSL and MRL of tomato plant was found statistically at par with treatment of *T. viride, B. subtilis and T. harzianum*. (Table-b-c). In soil B application of antagonist through root dip treatment were found more effective and increased the MSL and MRL of tomato plant *i.e.* 42cm & 9.6cm in *T. viride* 39cm & 8 cm in *B. sublitis* 30cm & 7.2cm in *T. harzianum*. While application of antagonists through soil inoculation in B soil also increases the MSL & MRL of tomato plant *i.e.* 40.40cm & 9.6cm in

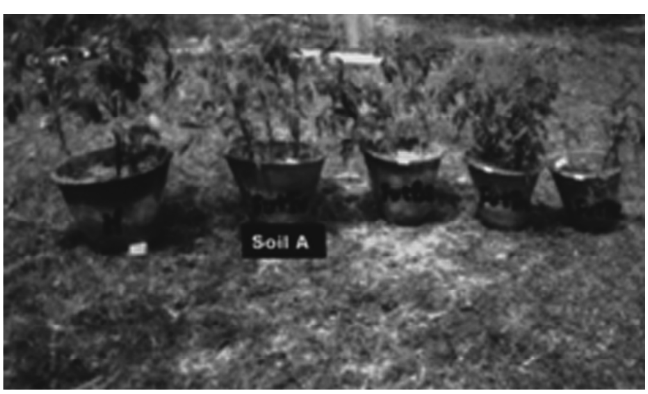

Figure 2: Application of antagonist in soil A

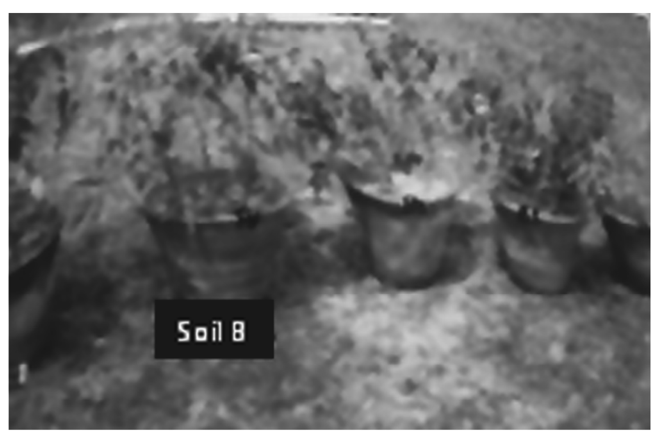

Figure 3: Application of Antagonist in Soil B

Table 3
Effect of Antagonist on the growth of tomato plant in Soil B (root dip and soil inoculation)

Treatment	*(MSL) (cm)*	*(MRL) (cm)*	*R% SL*	*R% RL*	*(MSL) (cm)*	*(MRL) (cm)*	*R% SL*	*R% SL*
	Root Dip				*Soil inoculation*			
Fo + Tv	42	9.6	18.80	14.20	40.40	9.60	21.90	14.21
Fo + Th	30	7.2	42	35.65	29.46	7.24	43.05	35.30
Fo + Tk	26.66	5.0	48.46	55.31	26.46	5	48.85	55.32
Fo + Bs	39.0	8.0	24.60	28.50	38.53	8.73	25.52	21.98
Control (C_1)	26	5.05	49.73	54.87	26	5.05	49.74	54.87
Control (C_2)	51.73	11.19			51	11.19		
SEd (±)	0.360	0.398			1.062	0.308		
CD (P=0.05)	0.720	0.796			2.124	0.616		
CD (P=0.01)	1.024	1.132			3.021	0.876		

Data are mean of 15 plants/treatment
Fo = *Fusarium oxysporum* f. sp. *lycopersici*
Th = *Trichoderma harzianum*
Tv = *T. viride*
Tk = *T. koningi*Bs = *Bacillus subtilis*

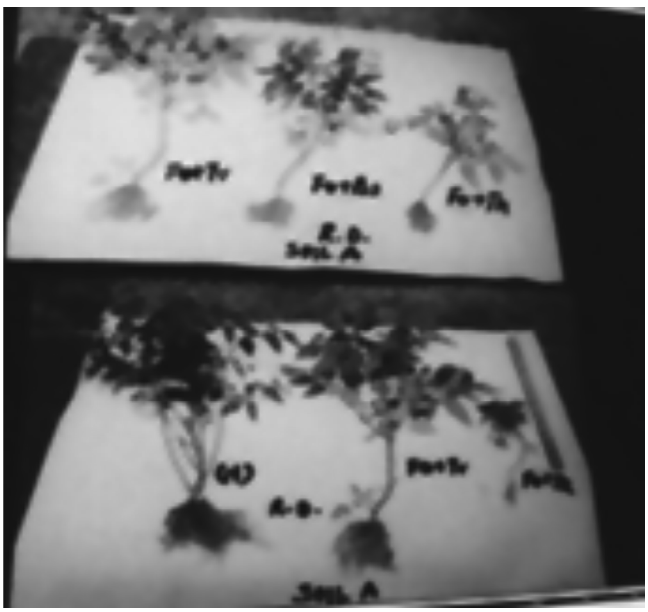

Figure 4: MSL & MRL of tomato plant in soil A (Root Dip)

T. viride, 38.53cm & 8.73cm in *B. subtilis*, 29.46cm & 7.24cm in *T. harzianum*. Application of antagonists in soil A and soil B through root dip treatment were found more effective than soil inoculation method . A considerable increase in MSL & MRL of tomato plant were observed by addition of *T. viride, B. subtilis and T. harzianum*. While in *T. koningi* no significant growth of tomato plant was observed (Table-b-c). Reduction percentage of shoot length and root length over uninoculated control showed a minimum reduction in *T.viride* and *B.subtilis* in both soil (Table-b). Raj & Kapoor (20) reported that use of

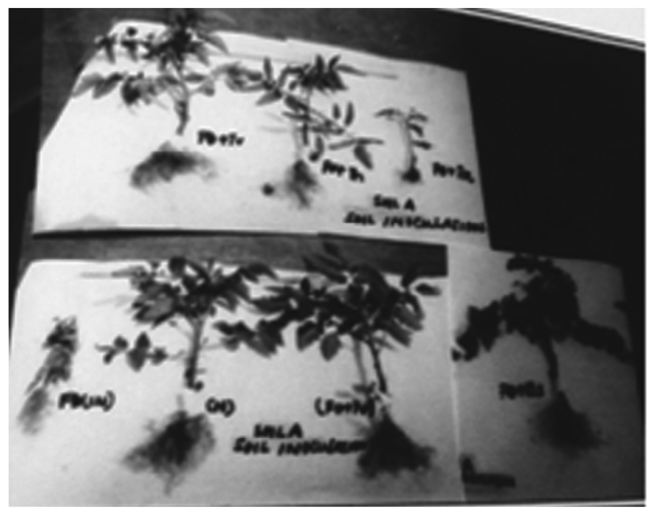

Figure 5: MSL & MRL of tomato plant in soilB (Root Dip)

cellulytic fungi (*Trichoderma* sp.) along with compost increases the shoot & root length of tomato plant. The increase in shoot length of muskmelon by application of *T. viride*was also reported by Chattopadhyay & Sen (4), Mohammad Akrami and Zohreh Yousefi (17). Similar observation was also reported by Schroth&Honcock (23). Gaikwad *et. al.*, (8) reported that *T. viride* and *T. harzianum* are strong antagonists which not only restricted linear growth and spore germination of *F.o.* f. sp. *Lycopersici* but also increased shoot & root length of tomato plant. Besides species of *Trichoderma* bacterial antagonists *B. subtilis*also increased the plant height, number of flowers, number of branches and root length in tomato plant as reported by Ghonim (11). Sarhan *et. al.*, (5), Gupta *et. al.*, (12). The results of the work

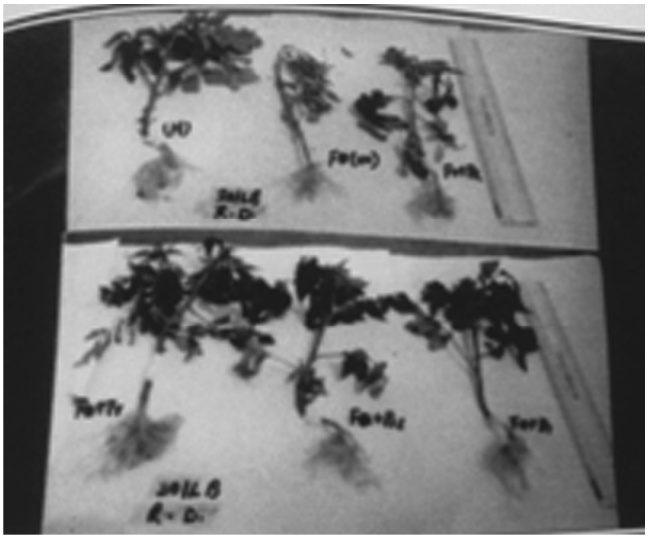

Figure 6: MSL & MRL of tomato plant in soil A(SI)

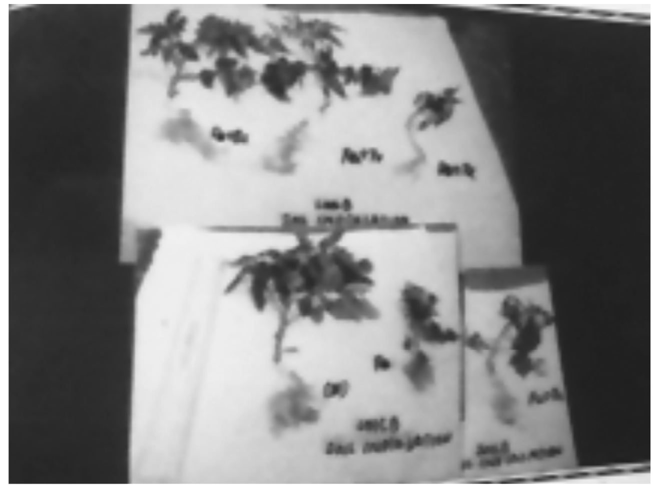

Figure 7: MSL & MRL of tomato plant in soil B(SI)

confirmed that antagonist *T. viride* and *B. subtilis* are more effective followed by *T. harzianum*. Similar findings were also reported by Chang *et. al.*, (5), Ao*et. al.* (1), Podile & Dube (18), Broadbent *et. al.*, (2), Canway & Kahn (6). Fresh and dry weight of tomato plant found considerably increased by application of antagonists *T. viride*, *B. subtilis*and *T. harzianum* in both Soil A & Soil B (Table) while no significant growth was observed in *T. koningi* as compared to control. Similar observation was also reported earlier by Ghonim (11). According to him, antagonist *B. subtilis*not only reduces the harmful effect of *F.o.* f. sp. *lycopersici*but also improve some growth parameters such as fresh and dry weight of tomato plant, height etc. Present observation also showed that use of *Trichoderma* sp. and *B. subtilis* not only inhibited the phytopathogen, but also increases the growth parameter of infected plant.

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Table 4
Effect of antagonists on biomass of tomato plant in Soil A & B

Treatment	*Soil A*				*Soil B*			
	Soil inoculation (SI)		*Root dip (RD)*		*Soil inoculation (SI)*		*Root dip(RD)*	
	Fresh weight (gm)	*Dry weight (gm)*	*Fresh weight (gm)*	*Dry weight (gm)*	*Fresh weight (gm)*	*Dry weight (gm)*	*Fresh weight (gm)*	*Dry weight (gm)*
Fo + Tv	16.83	6.13	18.80	7.054	16.20	6.05	16.72	6.90
Fo + Th	11.67	1.154	8.66	2.127	11.20	5.60	11.18	5.30
Fo + Tk	6.25	0.437	3.75	1.65	6.0	3.80	5.20	2.20
Fo + B	13.33	3.716	14	4.736	13.83	6.20	13.96	7.02
Control (inoculated Fo)	3.33	0.429	3.43	1.355	2.90	1.30	2.90	1.30
Healthy(Uninoculated)	22.66	9.024	22.66	9.024	22.60	8.02	22.60	8.02
SED (±)	0.349	0.030	0.428	0.709	0.636	0.701	0.903	0.530
CD (P = 0.05)	0.728	0.062	0.892	1.478	1.326	1.462	1.883	1.105
CD (P = 0.01)	0.992	0.085	1.217	2.017	1.809	1.994	2.569	1.507

Data are mean of 15 plants/treatment *Tk = T koningi*
Fo = Fusarium oxysporum f. sp.
Th = Trichoderma harzianum
Tv = *T. virideBs = Bacillussubtilis*

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