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Research Article

Evaluation of *Trichoderma* species against *Pythium ultimum* pathogenic to tomato

■ A.A. CHAVAN[1]*, G.D. DESHPANDE[2] AND S.N. ZAGADE[3]

[1]College of Agriculture, LATURE (M.S.) INDIA

[2]Department of Plant Pathology, Marathwada Agricultural University, PARBHANI (M.S.) INDIA

[3]Marathwada Agricultural University, PARBHANI (M.S.) INDIA

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*Corresponding author: pal_uasd@yahooo.in

ABSTRACT

Seedlings died of post emergence damping off were collected, isolations were made and pathogenicity was proved on Arka Vikas and Pusa Ruby cultivars. Re-ioslations were made from seedlings died after inoculation and original isolates were compared microscopically and found to be identical and therefore they were identified as *P. ultimum* Trow due to non-septate mycelium, inflated sparangia and thick walled oospores. Testing of biocontrol agents by daul culture method revealed that *Trichoderma harzianum, Trichoderm hamatum* and *Trichoderm konigii* significantly suppressed growth of *P. ultimum.*

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INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most economically important vegetable crop of India. India had 0.46 million ha under tomato with production of 8.4 million tonnes in 2000-01 (Anonymous, 2004). Economy of Rs.840 crore is involved in the tomato production of India. Out of this almost 9.7 to 10 per cent translocations of tomato economy occur in Maharashtra alone.

Commercially available tomato varieties face to the problem of poor germination due to pre-emergence damping off or seed rot caused by various *Pythium* species, in addition to succumbs to post emergence damping off causing seedling mortality from 25 to 100 per cent.

Different species of Pythium *viz., P. aphanidermatum, P. debaryanum, P. butleri* and *P. ultimum* have been recorded on tomato. Repeated isolations of affected tomato seedlings and rotten seeds yielded *P. ultimum* Trow. As pre and post-emergence damping off is noticed throughout Maharashtra and it is a major obstacle in the supply of quality seedlings in required quantity in the peak demand period of transplanting.

With the growing demand of tomatoes, it has become necessary to optimize the production and productivity of tomato by minimizing losses in by minimizing losses in diseases for reduction of losses in terms of seed rot and post emergence mortality, the present investigation on pre and post-emergence damping off in tomato were undertaken.

In soil there are many pathogens which cause seed rot. To identify the causal organism of the damping off, it is necessary to prove the pathogenicity of the isolated fungus.

Damping of disease is controlled with the use of bioagents *viz., Trichoderma* spp. To test the biocontrol agents, dual culture technique was used.

MATERIALS AND METHODS

The toppled seedlings of both the varieties were discoloured and rottened at the collar region. The toppled seedlings of both the varieties were washed in sterile water and then surface sterilized in 0.1 per cent $HgCl_2$ solution. After passing through three changes of sterile water, the collar bits were transferred to sterile PDA in Petri plates. After 48 hr, the mycelial growth appeared on collar region bits of both the varieties. The re-isolated cultures from both the varieties were transferred to PDA slants. The re-isolated cultures were compared with originally isolated culture from both the

varieties microscopically. The original and re-isolated culture were identical in both the varieties and was therefore identified as *Pythium ultimum* Trow.

Biocontrol agents act against pathogen either through parasitism, competition or antibiosis. In order to test the efficacy of some biocontrol agents, an experiment was conducted at Department of Plant Pathology, College of Agriculture, Latur in 2006. This experiment was conducted with following 8 treatments. For each treatment, 3 replicates of Petri plates were used. Standard PDA was prepared and sterile Petri plates were poured with sterile PDA near flame in isolation chamber. As per the requirement of treatment, 5 mm disc of either pathogen or antagonist (BCA) was inoculated at the centre in control Cp or C_A treatment. In dual culture treatments, the pathogen *P. ultimum* and antagonist (BCA) were inoculated with 5 mm inoculum disc at the distance of 45 mm. The observations on colony diameter of pathogen/ antagonists (BCA) were recorded after 5 days of incubation at room temperature (28 ± 5^0C).

RESULTS AND DISCUSSION

From Table 1 and 2, it can be concluded that only inoculated seedlings expressed mortality while control seedlings (I_0) did not show mortality indicating pathogenic nature of the culture on both the varieties used.

Seedlings of Arka Vikas and Pusa Ruby showing drooping, toppling and finally death of the seedlings yielded the same isolate. This isolate was found to be pathogenic by 100 (%) culture filtrate method as well as sick soil method on both the varieties. Re-isolations from dead seedlings from sick soil as well as from cultural filtrate yielded the same kind of pathogenic isolate which was used for preparation of sick soil or cultural filtrate. This clearly indicated that the original and reisolated cultures were pathogenic and caused post emergence damping off in Arka Vikas and Pusa Ruby cultivar. The microcopy of original and re-isolated culture also indicated that they were identical in morphology having non-septate mycelium, inflated sporangia and reproducing sexually by oospores. Therefore, original and re-isolated culture was identified as *Pythium ultimum* Trow. This culture was maintained on PDA slants and multiplied on PD Broth for further experimentation.

Pathogenic nature of *Pythium ultimum* Trow on tomato has also been reported by Bisht *et al.* (1997) from India Abdelzaher *et al.* (1997) from Egypt, Sinobas and Rodriquez (1999), Herreo *et al.* (2003) from Norway and Maulin *et al.* (1994). Present observations are in conformity with the above workers. However, in addition to *P. ultimum* Trow other species of *Pythium viz., Pythium aphanidermatum* (Brisht *et al.,* 19997; Herreo *et al.,* 2003; Moulin *et al.,* 1994), *P. irregulare* (Sinobas and Rodriquez, 1999, Herreo *et al.,* 2003 and Moulin *et al.,* 1994), *P. catenulatum* (Adelzaher *et al.,* 1997), *P. violae* (Abdelaher *et al.,* 1997), *P. paroecandrum* (Sinobas and Rodriquez, 1999; Herreo *et al.,* 2003) and *P. sylvaticum* (Moulin

Table 1 : Pathogencity on Arka Vikas and Pusa Ruby expressed as seedling mortality (%)

Sr. No.	Treatment key	Treatments	Mean mortality			Acrsin value
			Original value	$\sqrt{X+1}$ Transformation	$(\sqrt{X+1})^2$ value	
1.	V_1I_0	Arka Vikas control	0/16	1.00	1	0.5
2.	V_1I_1	Arka Vikas inoculated	6/16	5.26	40.96	30.46
3.	V_2I_0	Pusa Ruby control	0/16	1.00	1.0	0.50
4.	V_2I_1	Pusa Ruby inoculated	3/16	5.09	30.92	18.47
	V	V	I		V x I	
	S.E. $\pm$	5.62	5.62		7.96	
	C.D. (P=0.05)	17.31	17.31		24.49	

Table 2 : Interaction (V x I) expressed as seedling mortality in pathogenicity test

Sr. No.	Main treatment variety	Treat. Key	Sub-treatment		
			Seedling mortality		
			I_0 (Control)	I_1 (inoculated)	Mean
1.	Arka Vikas	V_1	0.5	30.4	15.5
2.	Pusa Ruby	V_2	0.5	18.4	9.5
	Mean		0.5	24.4	12.5
			V	I	V x I
	S.E. $\pm$		5.6	5.6	7.9
	C.D. (P=0.05)		17.3	17.3	24.4

Table 3 : Colony diameter of the pathogen as suppressed by different biocontrol agents

Sr. No.	Treatment code	Biocontrol agent/ pathogen	Colony diameter of *Pythium* (mm)	Colony diameter of antagonist (mm)	Mean
1.	BC_1+ P_0	*T. harzianum* alone	0.5	73.4	36.9
2.	BC_2+ P_0	*T. hamatum* alone	0.5	60.4	30.4
3.	BC_3+ P_0	*T. konigii* alone	0.5	72.4	36.4
4.	BC_4+ P_0	*T. lignorum* alone	0.5	78.9	39.7
5.	BC_0 + P_1	*Pythium* alone	89.9	0.5	45.2
6.	BC_1 + P_1u	*T. harzianum* + *Pythium ultimum*	44.6	3.6	24.1
7.	BC_2 + P_1u	*T. hamatum* + *Pythium ultimum*	45.6	3.6	24.6
8.	BC_3 + P_1u	*T. konigii* + *Pythium ultimum*	54.4	3.6	29.0
9.	BC_4 + P_1u	*T. lignorum* + *Pythium ultimum*	89.9	3.6	46.7
		Mean	26.6	37.5	33.4
		S.E. $\pm$	0.7	1.4	
		C.D. (P=0.05)	0.3	4.1	

et al., 1994) have been reported on tomato.

Among the all antagonists tested (Table 3), *T. harzianum* (BC_1) was significantly superior in suppressing the growth of *Pythium ultimum*, *T. harzianum* was followed by *T. hamatum* and *T. konigiii*. However, *T. lignorum* was significantly less effective.

Trichoderma hamatum (BC_2) has expressed unique property of inducing mycelial balls in *Pythium* which may be due to very sensitive nature of toxin produced by *T. hamatum* against *P. ultimum.*

Testing of various biocontrol agents by dual culture method revealed that *Trichoderma harzianum* was significantly superior over *T. hamatum* and *T. konigii. Trichoderma hamatum* and *T. konigiii* were significantly superior over *T. lignorum* in suppressing the growth of *Pythium ultimum* .

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