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Bioefficacy of Fungicides and *Trichoderma harzianum* against *Alternaria solani* Causing Early Blight of Tomato

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Abstract— *Early blight of tomato caused by Alternaria solani is the catastrophe disease worldwide. It causes threat both qualitatively and quantitatively to tomato cultivation. In the present investigation all the five fungicides viz. zineb, mancozeb, coppersulphate, thiram and carbendazim were in vitro screened against different strains of A. solani @ 500, 1000, 1500 ppm concentrations. Mancozeb was found most effective and achieved highest reduction in mycelial growth (86.4%). This was followed by thiram (82.7%), zineb (78.6%), copper sulphate (49.7%) and carbendazim (33.7%). All the fungicides tested caused significant inhibition at all the three concentrations tested over untreated control. Different strains of Trichoderma harzianum evaluated in vitro were found antifungal/ antagonistc against A.solani. T_5 was found to be the best strain of Trichoderma and recorded highest mycelial inhibition (79.1%) of the test pathogen. This was followed by T_2 (75.4%), T_3 (72.7%), T_1 (72.3%) and T_4 (70.6%). Under in vivo conditions foliar spray with mancozeb (0.2%) +Trichoderma harzianum (1×10^5 spore/ml) were highly effective in controlling the disease intensity as compared to their individual application.These results exhibit that assimilation of Trichoderma with moderate doses of the fungicides can offer beneficial control of A. solani. This will lower the reliance on the extensive use of fungicides leading to more environment friendly and cost effective control of the disease.*

Keywords: *Tomato, A.solani, T.harzianum ,mancozeb*

1. INTRODUCTION

Tomato (Lycopersicon esculentum) is an important vegetable worldwide. *Alternaria solani* causes early blight disease that has become most destructive in all over the world and yield losses up to 15- 100 % [1]. It is responsible for the deterioration of quality and quantity of tomato yield [2]. The disease is controlled mainly by the application of synthetic chemicals which are very much hazardous for the environment and human health. Recently, efforts in different parts of world are made to develop environmentally safe methods for reducing the use of agrochemicals. The development of resistant strains of *A.solani* and increased fungicide residues has given a thrust to plant pathologists to search for natural products for substituting synthetic fungicides.

Literature survey indicated several antagonistic strains of *T.harzianum* as effective biocontrol agents against *Alternaria* species. The present study aims to evaluate *T. harzianum* and compare their efficacy with most effective fungicides employed in the management of early blight disease.

2. MATERIAL AND METHODS

2.1. Isolation and preparation of pure culture.

The different isolates of *A.solani* viz. A_1, A_2, A_3, A_4, A_5 used in the study were obtained from infected tomato leaves following single spore isolation techniques. The pure culture of isolates were tested for pathogencity and maintained on PDA medium for further investigations.

2.2. Evaluation of fungicides against early blight of tomato.

The efficacy of fungicides *viz.* zineb, mancozeb, coppersulphate, thiram and carbendazim against different strains of *A.solani* were evaluated *in vitro* by food poison technique using PDA as basal medium. The observation of radial growth of *A.solani* was recorded on 8^{th} day when maximum growth was observed in control.

The percent inhibition was calculated and the data was analyzed statistically [3].

$$PI = C - T / C \times 100$$

I = Percent inhibition; C = mycelial growth in control; T = mycelial growth in treated plates

2.3. Evaluation of *T.harzianum* against early blight of tomato.

The antagonistic effect of different strains of *T.harzianum* *viz.*T_1, T_2, T_3, T_4, T_5 were evaluated by dual culture technique on PDA medium [4]. Mycelial growth of different isolates of *A.solani* were recorded after four days of inoculation. The percent inhibition of isolates of *A.solani* were calculated and the data was analyzed statistically.

2.4. Evaluation of fungicides and *T. harzianum* against early blight of tomato under green house conditions.

The fungicides and *T.harzianum* were evaluated *in vivo* against *A.solani*. In case of fungicides 0.2% concentration was used while *T.harzianum* at spore suspension of 1×10^5 spores/ml was used. The experiment was laid out in Randomized block design with three treatments. The treatment consists of mancozeb (F.S.), *T. harzianum* (F.S.), mancozeb + *T.harzianum* (F.S.), Control; F.S. = foliar spray.

The application of the first spray was done at the initiation of the disease symptom and second after 15 days up to 60 days after planting. In case of control infected plants were sprayed with water. Percent disease index was calculated following the given formulae [5]

$$PDI = \Sigma (n \times v) / N \times S \times 100$$

Where, Σ = summation; N = no. of leaves in each category; V = numerical value of leaves observed; S = maximum numerical grade.

2.5. Statistical analysis.

At the end of the laboratory and pot experiments the data on the effect of the treatments on the growth of *A.solani* was analysed by One Way ANOVA and treatment means were compared by Fishers least significant difference procedure (LSD) at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of fungicides on Inhibition of *A.solani.*

Five fungicides were selected and evaluated against the different strains of *A.solani.* Results [Fig.1] revealed that all the fungicides (@ 500, 1000, 1500 ppm) were effective in inhibiting the mycelial growth of *A.solani* over untreated control. Increase in the concentration of fungicides tested increased the percent mycelial inhibition of test pathogen. The mancozeb, thiram and zineb caused the highest mycelial inhibition of *A. solani* (86.4%, 82.7% and 78.6%). copper sulphate and carbendazim caused lowest inhibition (49.7%, 33.7%) in mycelial growth of *A. solani*. Mancozeb recorded highest mycelial inhibition in A1 A3 and A5 (86.4%, 72.4%, 79.8%) strains of *A.solani* presented in Table 1, 3, 5 and figure1. Fungicides thiram and zineb recorded highest mycelial inhibition in A2 and A4 (82.7%, 78.6%) strains of *A.solani* presented in Table 2, 4. Similar fungistatic effects of different fungicides against *A.solani* infecting tomato were reported earlier by several workers [6].

Table 1: Efficacy of different fungicides against *Alternaria solani* isolates A_1.

Fungicides	Colony Dia.(mm)			Mean		Percent Inhibition		Mean
	500	1000	1500	(mm)	500	1000	1500	%
Mancozeb	18	12.6	5.3	12.0	79.5	85.6	94	86.4
Thiram	27	22.3	19	22.8	69.2	74.6	78.7	74.2
Copper sulphate	65	56.6	44.6	55.4	25.8	35.6	50	37.1
Carbendazim	72.3	65	57.6	65.0	17.5	26.1	35.4	26.3
Zineb	34	28	21.3	27.8	61.2	68.2	76.1	68.5
Control	87.6	88	89.3	88.3	0	0	0	-
SE±(m)	0.077	0.07	0.067	-	0.10	0.06	0.07	-
SE±(d)	0.109	0.099	0.094	-	0.15	0.09	0.10	-
CD 1%	0.234	0.211	0.202	-	0.31	0.19	0.22	-

Average of three replications
Figures in parenthesis are arc sine values

Table 2: Efficacy of different fungicides against *Alternaria solani* isolates A_2.

Fungicides	Colony Dia.(mm)			Mean		Percent Inhibition		Mean
	500	1000	1500	(mm)	500	1000	1500	%
Mancozeb	23.3	17.6	14.3	18.4	73.4	80	83.6	79
Thiram	20.6	14.6	10.3	15.2	76.4	83.4	88.2	82.7
Copper sulphate	77.6	62.6	58	66.1	11.4	29.1	33.6	24.7
Carbendazim	67.3	58	49.2	58.2	23.2	34.3	43.6	33.7
Zineb	36.6	27.3	20.3	28.1	58.2	69.1	76.7	68.0
Control	87.6	88.3	87.3	-	0	0	0	-
SE±(m)	0.087	0.079	0.08	-	0.11	0.09	0.10	-
SE±(d)	0.124	0.112	0.113	-	0.15	0.13	0.14	-
CD 1%	0.264	0.239	0.242	-	0.33	0.28	0.30	-

Average of three replications
Figures in parenthesis are arc sine values

Table 3: Efficacy of different fungicides against *Alternaria solani* isolates A_3.

Fungicides	Colony Dia.(mm)			Mean	Percent Inhibition			Mean
	500	1000	1500	(mm)	500	1000	1500	%
Mancozeb	33.6	24.3	16	24.6	62.5	72.6	82.1	72.4
Thiram	55.6	46.5	25	42.4	37.9	47.7	72	52.5
Copper sulphate	73.2	67.3	59	66.5	18.3	24.3	34	25.5
Carbendazim	71.5	64	50.3	61.9	20.2	28	43.7	30.6
Zineb	66	55.2	37	52.7	26.3	37.9	58.6	40.9
Control	89.6	89	89.5	89.4	0	0	0	-
SE±(m)	0.079	0.074	0.609	-	0.08	0.07	0.07	-
SE±(d)	0.112	0.104	0.097	-	0.12	0.10	0.09	-
CD 1%	0.239	0.223	0.208	-	0.25	0.21	0.20	-

Average of three replications
Figures in parenthesis are arc sine values

Table 4: Efficacy of different fungicides against *Alternaria solani* isolates A_4.

Fungicides	Colony Dia.(mm)			Mean	Percent Inhibition			Mean
	500	1000	1500	(mm)	500	1000	1500	%
Mancozeb	46.2	35.3	24	35.2	46.8	59.6	72.7	59.7
Thiram	33.3	26.6	20.3	26.7	61.7	69.5	76.9	69.4
Copper sulphate	63.3	52.4	31.6	49.1	27.2	40	64	43.7
Carbendazim	75.2	63.3	47	61.8	13.5	27.5	46.6	29.2
Zineb	24.6	18.6	12.6	18.6	71.7	78.6	85.6	78.6
Control	87	87.3	88	87.4	0	0	0	-
SE±(m)	0.085	0.081	0.091	-	0.12	0.10	0.08	-
SE±(d)	0.12	0.114	0.129	-	0.17	0.14	0.11	-
CD 1%	0.257	0.244	0.276	-	0.37	0.31	0.23	-

Average of three replications
Figures in parenthesis are arc sine

Table 5: Efficacy of different fungicides against *Alternaria solani* isolates A_5.

Fungicides	Colony Dia.(mm)			Mean	Percent Inhibition			Mean
	500	1000	1500	(mm)	500	1000	1500	%
Mancozeb	26.5	17.6	8.6	17.6	69.3	79.9	90.1	79.8
Thiram	37.6	30.3	22.3	30.1	56.5	65.4	74.4	65.4
Copper sulphate	58	42.7	30.6	43.8	33.1	51.2	64.9	49.7
Carbendazim	73.6	69	56	66.2	15	21.3	35.9	24.1
Zineb	32.3	26.3	17.3	25.3	62.7	70	80.2	71.0
Control	86.6	87.6	87.3	87.2	0	0	0	-
SE±(m)	0.08	0.05	0.07	-	0.08	0.06	0.08	-
SE±(d)	0.11	0.08	0.1	-	0.12	0.09	0.11	-
CD 1%	0.24	0.17	0.23	-	0.25	0.19	0.24	-

Average of three replications
Figures in parenthesis are arc sine

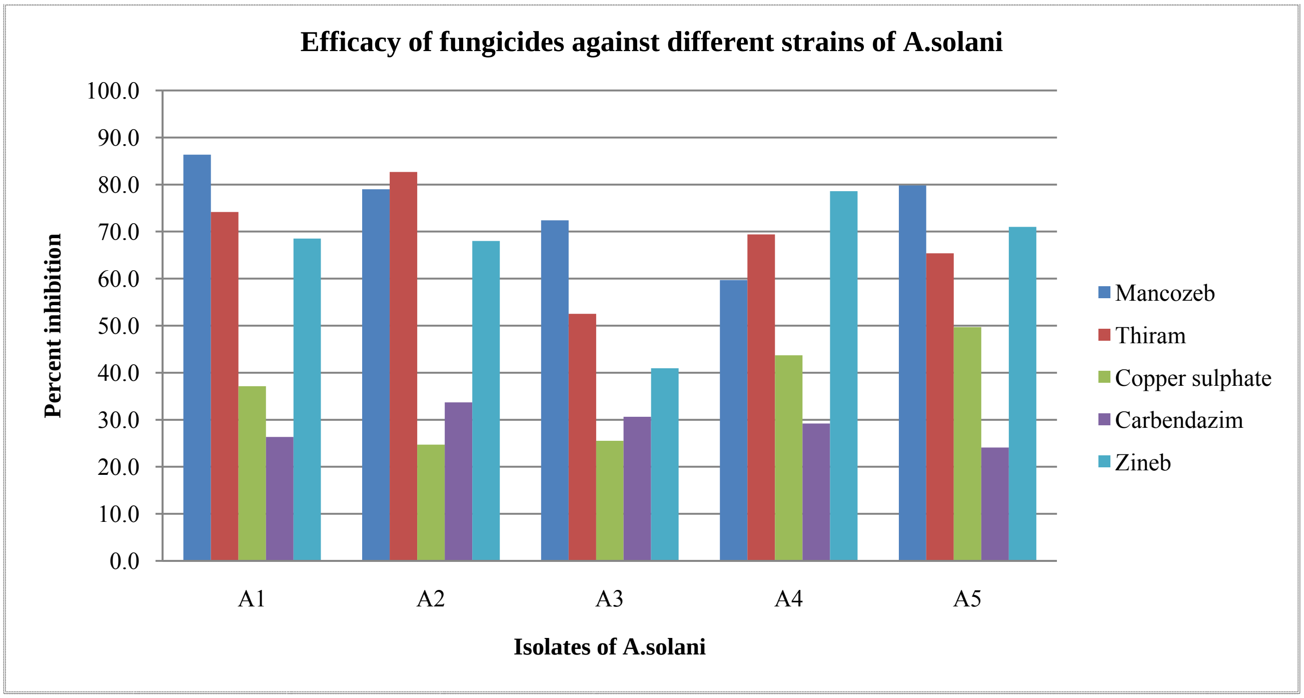

Fig. 1: Efficacy of fungicides against different strains of *A.solani in vitro*

3.2. Effect of different strains of *T. harzianum* on inhibition of different isolates of *A.solani*

All the different strains of *T. harzianum* evaluated exhibited antagonistic / antifungal activity against different strains of *A.solani* and significantly inhibited radial growth of mycelium over untreated control. T_5 exhibited maximum mycelial inhibition (79.1 %) followed by T_2, T_3, T_1 and T_4 (75.4%,72.7%, 72.3% and 70.6%) as presented in Table 6 and figure 2.The inhibition of different isolates of *A.solani* growth by antagonistic *T. harzianum* isolates suggested the strong mycoparasitic activity by the production of inhibitory substances which inhibit the sporulation of the pathogen . Results of the present study of bioagents *viz. T. harzianum* strains against *A.solani* are in conformity with those reported by several authors [6],[7],[8].

Table 6: Evaluation of Colony diameter and percent inhibition of different strains of *T. harzianum* against different isolates of *A.solani*

Bioagents	Colony diameter					Percent inhibition					Mean
	A1	A2	A3	A4	A5	A1	A2	A3	A4	A5	
T1	26.7	16.2	21	36	19.3	68.9	80.0	76.6	57.9	78.1	72.3
T2	13.6	17.3	25.6	23.5	26	84.1	78.7	71.4	72.4	70.6	75.44
T3	23.7	24.3	27.3	25	16.6	72.4	70.0	69.5	70.8	81.1	72.76
T4	25.3	21.4	32	9.3	39	70.5	73.6	64.3	89.1	55.9	70.68
T5	21	25.2	17	17	0.8	75.5	68.9	81.0	80.1	90.2	79.14
control	85.8	81.1	89.6	85.6	8.83	0	0	0	0	0	
SE(±m)	0.1	0.09	0.094	0.12	0.10	0.12	0.08	0.10	0.13	0.12	
SE(±d)	0.14	0.13	0.133	0.17	0.15	0.17	0.11	0.14	0.19	0.17	
CD 1%	0.32	0.29	0.294	0.37	0.32	0.37	0.23	0.32	0.42	0.37	

Average of three replications
Figures in parenthesis are arc sine values

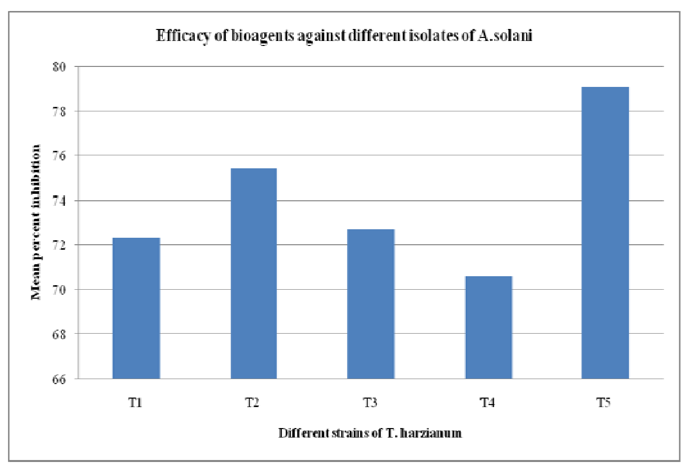

Fig. 2: Efficacy of different strains of *T. harzianum* against *A.solani* isolates.

3.3. Effect of fungicides and *T. harzianum* against early blight of tomato under green house conditions

The fungicides and *T.harzianum* were assessed under green house conditions against early blight disease of tomato. The treatments comprised of mancozeb at 0.2%, T_5 at 1×10^5 spore/ml and mancozeb + T5. Observations on disease severity were recorded 15 days after last spray. All the treatments significantly reduced the disease as evident from the data presented in Table 7.Maximum disease reduction was observed in plants treated with foliar spray with mancozeb + foliar spray with T_5 (79.1 %) as compared to foliar spray with mancozeb (66.6 %) and foliar spray with T_5 (58.8%) separately. The integration of biocontrol agents and fungitoxicants showed significant reduction in the early blight disease infection and improved the yield of tomato plant as compared to infected control. In form of spray bioagents are used *in vivo* to inactivate the pathogen spores as they land on plant surface [9]. These results were similar to the earlier work of bioagents and fungicides [10].

In conclusion our study demonstrated that *T.harzianum* can be used in combination with minimum concentration of fungicides for the control of the early blight disease. Thus, this method can minimize the risk hazards in use of toxic fungicides and develop environmentally safe alternatives for the management of blight disease.

Table 7: Evaluation of mancozeb, T_5 and their combinations against early blight of tomato under green house conditions.

Treatments	Isolates of *A.solani*	disease severity	reduction (%)	Mean
Mancozeb	A1	15.6	74.8	
	A2	21.3	64	
	A3	19.2	70	66.6
	A4	31.2	49.1	
	A5	14.3	75.4	
T_5	A1	24.8	60	
	A2	28.3	52.1	
	A3	22.1	65.5	58.8
	A4	32.4	47.1	
	A5	17.8	69.4	
Mancozeb + T_5	A1	7.2	88.4	
	A2	16.9	71.4	
	A3	9.3	85.4	79.1
	A4	19.2	68.6	
	A5	11.4	82.1	
Control	A1	62.1	0	
	A2	59.2	0	
	A3	64.1	0	
	A4	61.3	0	
	A5	58.2	0	
CD (p=0.05)	A1	0.492	1.413	
	A2	0.74	1.867	
	A3	2.41	2.277	
	A4	3.43	1.39	
	A5	2.435	1.24	
SE±(m)	A1	0.14	0.4	
	A2	0.21	0.529	
	A3	0.683	0.646	
	A4	0.972	0.394	
	A5	0.69	0.352	
SE±(d)	A1	0.197	0.566	
	A2	0.297	0.748	
	A3	0.966	0.913	
	A4	1.375	0.557	

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