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STUDIES ON DIFFERENT FUNGICIDES, BIOCONTROL AGENTS OF EARLY BLIGHT OF TOMATO CAUSED BY *ALTERNARIA SOLANI*

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Abstract– A significant vegetable crop that is utilized both as a vegetable and as an ingredient is the tomato (*Solanum lycopersicum* L.). Early blight in tomato caused by *Alternaria solani* which is responsible for significant yield losses in the crop. Several of these techniques have been evaluated against *A. solani* as part of the current investigation. Two biocontrol agents and five fungicides were evaluated following poison food technique and dual culture. At the highest concentration (75 ppm) of fungicide, the least average mycelial growth of *Alternaria solani* was shown by Azoxystrobin 18.2%+difenoconazole 11.4% SC (20.01mm) followed by Propiconazole 25% EC (25.20mm), Mancozeb75% WP (39.50 mm), Chlorothalonil 75%WP (44.63mm) and Copper oxychloride 50%WP (49.50 mm). At concentration, i.e. 75 ppm, Azoxystrobin 18.2% + difenoconazole 11.4% SC showed maximum inhibition of mycelial growth (77.76%), followed by Propiconazole 25% EC (72.00%), Mancozeb 75% WP (56.11 %) and Chlorothalonil 75%WP (52.77 %). The pathogen was inhibited the least in copper oxychloride 50 percent WP (47.25 percent). The minimum colony growth of *Alternaria solani* was observed in *Trichoderma viride* (23.60 mm) followed by *T. harzianum* (18.60 mm). After 7 days maximum inhibition was observed in mycelial growth of (79.33%) shown by *T. harzianum* followed by *T. viride* (73.77%). Similar trend was also observed in field conditions. Among different fungicides, Azoxystrobin + difenoconazole (15.5%) showed less disease severity as compared to other fungicides. Both biocontrol agents showing less diseases control percentage (61.10 to 65.74%) with diseases severity (15.5 to 17.6%) and significantly enhanced plant growth parameter and fruit yield.

INTRODUCTIION

Tomato (*Solanum lycopersicum* L.) is one of the most remunerable and widely grown vegetable in the world belongs to family solanaceae. China is the leading country in production of tomato (31%), followed by India and United states with the second and third highest producers in the world (Bais *et al.,* 2019). The consumption of tomato stands second after potato being rich in vitamins (K, C and A), minerals (Fe, Ca and P), amino acids, sugars, dietary fibres and antioxidant and contains 95.3% of water (Bhanage *et al.*, 2019). Due to the attack of numerous bacterial, viral, and fungal diseases, the production of tomatoes facing a significant loss in production (Devi *et al.*,2017). Fusarium wilt, early blight, damping off, late blight, tomato mosaic virus, verticillium wilt, and bacterial wilt are the main diseases in tomato crop (Rex *et al.,* 2019). Early blight, one of the important tomato diseases brought on by the pathogenic fungus *A. solani*, is the most detrimental of all of them and significantly reduces both the amount and quality of fruit yield (Tomazoni *et al.,* 2017, Chohan *et al.,* 2019).).Firstly appears on older leaves and then spread infection on all the above ground parts of tomato (stems, petiole, twig and fruits). Under favourable conditions, defoliation, drying off of twigs and premature fruit drop can occur inflicting losses from 50 to 86 per cent in fruit yield (Shoaib *et al.,*2019, Bais *et al.,* 2019).Various controls have been implemented to manage and better treat this disease.As some workers have pointed out, the use of fungicides is considered to be the most effective way to control early blight (Egel *et al.,* 2019; Farooq *et al.,* 2019). Biological pest control agents are also recognized as an effective and ecological alternative approach to combat early blight. Biological pest control agents have proven to be effective and environmentally friendly. In addition, they are easily biodegradable,

non-phytotoxic, systemic and environmentally safe (Devi *et al.,* 2017, Chanthini *et al.,* 2018; Youssef *et al.,* 2018).

MATERIALS AND METHODS

All the experiments were conducted *in vitro* at Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara, Punjab. The fungicides used in this research were purchased from local market. Bio controls *Trichoderma harzianum* and *T. viride* were obtained as pure culture from our university Lab.

Isolation and identification of *Alternaria solani*

The pathogen was secluded from early blight infected leaves. Infected leaves were washed with tap water to remove the soil and then dried on the blotting paper and were then cut into small pieces sterilized with 0.1% Sodium hypochlorite solution (NaClO) for 30 seconds. After that, the bits were again washed with distilled water thrice to remove sodium hypochlorite solution. They were then arranged on petri plates containing potato dextrose agar (20 ml PDA) aseptically in laminar air flow. The petri plates were incubated at (26 ±1°C) for 7 days and were observed for sporulation and fungal growth. Colonies were observed under compound microscope on the basis of mycelial growth and spore characters. They were sub cultured on PDA after identification and maintained for future use.

In vitro efficiency of fungicides on mycelial growth of *Alternaria solani*

In this bioassay demonstration the technique called poisoned food technique was used to check of different fungicides. Five fungicides such as Copperoxychloride, Azoxystrobin, difenoconazole, propicanozole and mancozeb, cholorothalonil were used. These fungicides were used at three different concentrations (25,50,75 ppm). Sterilized PDA media was kept in laminar air flow. 60 ml PDA media was poured in 1st flask and 100 ppm fungicide was added into it. Then 60 ml of PDA was added into 2nd flask and 200 ppm fungicide was added into it. Again 60 ml PDA was added into 3rd flask and 500 ppm fungicide was added into it. These three flasks were further poured into Petri plates. Each flask was poured in 3 Petri plates and then 5 mm disc of pathogen is placed in Petri plate, these Petri plates were then kept in an incubator at 26±1 °C. The activity of fungicides was recorded after 7 days when the control plate was fully grown. Below equation is used to calculate the per cent growth inhibition of the fungus in each treatment in comparison with control (Vincent, 1972).

$$PI=(C-T)/Cx100$$

Where,
PI= Percent inhibition
C= Radial growth in check in mm
T= Radial growth in treated plates in mm

Efficacy of bio-control agents (*In-vitro)* on mycelial growth of *Alternaria solani*

The pure cultures of *T. harzianum* (ITCC: 5593) and *T.viride* (ITCC: 6315) were obtained from the Indian Type Culture Collection (ITCC) available in Division of Plant Pathology, ICAR- IARI, New Delhi and cultures were grown in potato dextrose agar (PDA) plates and stored at 4 °C for further use. The efficiency of two bio agents (*T. harzianum* and *T. viride*) was assessed against *A. solani* for radial growth inhibition on the PDA medium. The PDA medium was used to grow pathogen and test organism separately for that PDA was poured into 90 mm diameter Petri dishes permitted to solidify. Seven days old culture of *A. solani* (5 mm disc)was placed at one end of Petri dish and respective antagonistic organisms were inoculated at the opposite side. Petri plates without antagonist served as control. The activity of bio control agents were recorded after 7 days when the control plate was fully grown. Below equation is used to calculate the per cent growth inhibition of the fungus in each treatment in comparison with control

$$PI=(C-T)/Cx100$$

Where,
PI= Percent mycelial growth inhibition
C= Colony diameter in control in mm
T= Colony diameter in respective treatment (mm)

Evaluation of chemical fungicides and biocontrol agents against *Alternaria spp* under *in vivo* (Field condition)

Field trial demonstration was conducted in Lovely Professional University agriculture farm in which the different selected chemical fungicides and biocontrol agents against tomato early blight caused by *Alternaria* spp in the experimental plot. The tomato variety used was cs990. The plants were transplanted at recommended spacing (60x45cm)in Randomised Block Design (RBD) with three replications and 8 treatments in 500 square metre area. Examining the symptoms appeared on leaves

and recorded disease severity and fruit yield per plot. Disease severity data was recorded before spray as initial, 10 days after 1st,2nd and 3rd spray. Early blight disease severity was recorded on 10 plants were selected at randomly in each replication of the treatment. The Fruit yields were recorded after harvesting the crop at maturity and expressed in Q/ha.

PDI=Sum of numerical values/no of leaves observed ×100/Maximum rating scale

Rating scale for assessment of early blight disease (Mayee and Datar, 1986)

Grade	Description of the symptoms
0	No symptoms on the leaf
1	0-5 per cent leaf area infected and covered by spot on leaf
2	6-20 per cent leaf area infected and covered by spot, some spots on petiole
3	1-40 per cent leaf area infected and covered by spot, spots also seen on petiole, branches
4	41-70 per cent leaf area infected and covered by spot, spots also seen on petiole, braches, stem
5	>71 per cent leaf area infected and covered by spot, spots also seen on petiole, branch, stem, fruits

RESULTS AND DISCUSSION

Mycelial growth of *Alternaria solani* (*In vitro*) by using different bio-controls

Dual Culture: The antagonistic activity of bio agents against *A. solani* was observed through Dual Culture Technique on PDA. The results presented in Table 1, revealed that all the two species of *Trichoderma* (*T. harzianum* and *Trichoderma viride*) showed significantly different antagonistic potential against *A. solani*.The average minimum colony growth of *Alternaria solani* was observed in *T. harzianum* (18.6 mm) followed by *T. viride* (23.60 mm). Highest inhibitory effect was observed after 7 days with maximum inhibition of mycelial growth (79.33 %) shown by *T. harzianum* (Plate 1) followed by *T.viride* showed less inhibition (73.77%) (Plate 2).

The results were supported by findings of Ganie *et al.* (2013) evaluated some bioagents against *Alternaria solani* through dual culture technique and maximum mycelial growth inhibition of *Alternaria solani* was observed in the case of *Trichoderma harzianum* (71.85%), followed by *Trichoderma viride* (65.93%) and *Trichoderma virens* (58.65%). It is also proved by Vaibhav Pratap Singh *et al.* (2018) evaluated seven fungicides along with seven biocontrol agents and seven plant extracts in *In vitro* condition against *Alternaria solani* and in this experiment *Trichoderma harzianum* was found to be effective and recorded highest inhibition (80.37%) of the mycelial growth of the pathogen followed by *T. viride* (77.41%), *T. koningii* (71.48%) and least mycelial inhibition shown by *T. hamatum* (27.41%). Related type of results was alsoreported by Koley *et al*. (2015). Mahantesh *et al*. (2017), also reported maximum inhibitory effect shown by *T.harzianum* (80.36%). Devi *et al*. (2017), reported that *T. harzianum* and *T. hamatum* showed 100 % inhibitory effect on growth and sporulation of *A. solani*. Chohan *et al*., (2015), reported that *Trichoderma harzianum* could inhibit 67.78% on the growth of *A. solani* whereas *Trichoderma viride* showed less inhibition (59.63%). Naik *et al* (2020) also found that the maximum inhibition was obtained by *Trichoderma harzianum* (85.13%) and *Trichoderma viride* (80.67%) growth inhibition over all other biocontrol agents.

Table 1. Inhibitory effect of bio-control agents on growth of *Alternaria* spp by using dual culture technique.

Treatments	Mycelial growth (mm)	Percent inhibition (%)
Trichoderma viride	23.60	73.77
Trichoderma harzianum	18.60	79.33
Control	90.0	-
C.D value	1.233	-
S.E (m)	0.350	-
S.E(d)	0.494	-
C.V value	1.374	-

In vitro efficacy of fungicides against *Alternaria solani*

The fungi toxic effects of five fungicides at three different concentrations were evaluated *in vitro* against *A. solani* by using Poison Food technique. The results showed that with the increase in fungicide concentration, the mycelial growth of *Alternaria solani* was significantly decreasing when compared with control (Table 2). Among the fungicides used, at 75 ppm the least mycelial growth was shown by Azoxystrobin+difenoconazole 18.2%+11.4% SC (20.01 mm) followed by Propiconazole 25%EC (25.20 mm), Mancozeb 75%

WP (39.50 mm), Chlorothalonil 75% WP (42.50 mm) and Copper oxychloride 50% WP (47.25 mm).

Mycelial Inhibition: The results showed that all fungicides inhibit mycelial growth of the *A. solani*, when compared with control (Table 2). At 75 ppm, the highest percent inhibition were observed in Azoxystrobin+difenoconazole 18.2%+11.4% SC (77.76%) followed by Propiconazole 25% EC (72%), Mancozeb 75% WP (56.11 %), Chlorothalonil 75%WP (52.77 %) and Copper oxychloride 50% WP (47.5 %).

Many researchers have reported the effectiveness of different fungicides against the mycelial growth of *A. solani*. The use of fungicides is considered as the most effective approach for controlling early blight (Egel *et al*., 2019; Farooq *et al*., 2019). The fungicide Mancozeb has been widely used since the last two decades to control early blight, reducing the severity of the disease and enhance tomato yield (Bais *et al*., 2019).

The findings were supported by Hassan *et al*. (2014) determined the efficacy of commonly available fungicides at six different concentrations against *Alternaria solani*. Results showed that Chlorothalonil has better effectiveness as compared to others followed by Clipper and Antracol.These findings are consistent with those reported previously by several workers. Fungicides such as hexaconazole 4% + zineb 68 %, pyraclostrobin 133 G/L + epoxiconazole 50 G/L SE, difenoconazole 25 EC, trifloxystrobin 25% + tebuconazole 50% have been shown to significantly inhibit *Alternaria solani* and *A. alternata* (Herle and Kamanna, (2014); Kumar and Singh (2017); Sharma *et al*., (2018) and Sudarshan *et al*., (2020). Similar results were found for fungicides such as azoxystrobin 23 SC by Kumar and Singh (2017); Thejakumar and Devappa (2016) and Deshmukh (2017). Rani *et al*, (2017) reported that Tebuconazole and Difenoconazole were most effective fungicide in controlling early blight of tomato. While according to Farooq *et al*, (2019) reported that Hexaconazole and Pyraclostrobin are the best fungicides to reduce the occurrence of the disease by inhibiting maximum growth of mycelium. Deshmukh *et al*. (2020) tested six fungicides, most effective fungicides were found Mancozeb 75% WP which exhibited 100% inhibition in mycelium growth at 0.2% concentration followed by Tebuconazole 50% + Trifloxystrobin 25% w/w (75WG) 85% inhibition in mycelium growth at 0.1% concentration.

Effect of *Trichoderma* spp. against *Alternaria solani* pathogen under field experiment

The effect of selected highly antagonistic isolates of *Trichoderma* spp. (*T. harzianum and Trichoderma viride*)

Table 2. Fungicides showing inhibition percent against *Alternaria* spp under *in vitro* condition.

Treatments	Concentration (ppm)	Mycelial growth (mm)	Percent inhibition%
Azoxystroblin + difenconazole	25	27.1	69.88
	50	23.5	73.88
	75	20.01	77.76
Propiconazole	25	35.5	60.55
	50	30.25	66.38
	75	25.2	72
Mancozeb	25	45.5	49.44
	50	40.5	55
	75	39.5	56.11
Cholorathional	25	50.5	43.88
	50	46.75	48.0
	75	42.5	52.77
Copper oxychloride	25	55.5	38.33
	50	50.25	44.16
	75	47.25	47.5
Control	25	90.0	0
	50	90.0	0
	75	90.0	0
C.D value		6.315	
S.E(m) value		2.027	
S.E(d) value		2.867	
C.V value		7.411	

soil on the pathogenic fungi (Table 3) was found effective in suppressing early blight disease incidence of tomato compared with control (60%). Notably, the application of *T. harzianum* to infested soil caused the highest reduction in disease severity percentage (25.07%) followed by *Trichoderma viride* (27.41%).

Efficacy of fungicides against *Alternaria solani* under field conditions

Results revealed that the PDI was in the range of 20.50 to 33.50% in the experimental plots after giving the first fungicidal application. These ranges in the disease incidence were differing significantly in the plots meant for different treatment. But in subsequent sprays, all the fungicides treated plots recorded significantly less severity disease severity over control on different days of observation. The data on percent disease severity of early blight of tomato are presented in Table 3. Results clearly revealed that, the foliar spray of Azoxystrobin 18.2%+ difenoconazole 11.4%SC (2.5 ml /l) dosage (terminal PDI, 15.50%) provided the least incidence of the early blight. In the control plot, percent disease severity was as high as 45.25 per cent. However, maximum per cent disease control (66.62%) was recorded after 3rd foliar sprays in the treatment Azoxystrobin 18.2% ++ difenoconazole 11.4% SC (2.5 ml/l).

Experimental findings shown that terminal

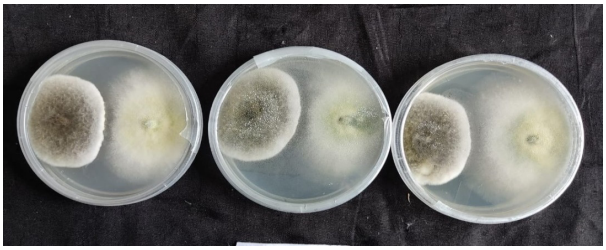

Plate 1. Dual culture of *Trichoderma viride* against *Alternaria solani*

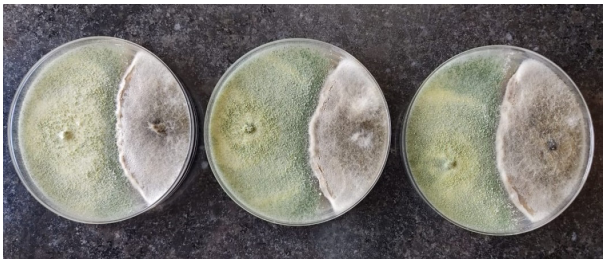

Plate 2. Dual culture of *Trichoderma harzianum* against *Alternaria solani*

Table 3. Effect of different treatments on Percent Disease Intensity of early blight of tomato under field conditions

Treatments & Doses	Percent Disease Intensity %			Disease control %	Shoot length cm	Root length cm	No. of fruits/ plant	Plant height (cm)	Yield (q/ha)
	Initial days of spraying	After spray of 35 days	After spray of 45 days						
Trichoderma harzianum (125 g/l)	25.5	19.1	15.5	65.74	46.47	34.13	19	57.66	48.6
Trichoderma viride (125 g/l)	20.5	19.5	17.6	61.1	45.58	32.77	18	54.66	36
Azoxystrobuin+difenoconazole (2.5 ml/l)	21.3	18.8	15.1	66.62	42.61	25.55	16	54	42
Propiconazole (1 ml/l)	26.9	18.5	17.5	61.32	34.49	25.36	15	51.33	38
Mancozeb (2.67 g/l)	27.6	25.4	22.1	51.16	44.42	28.55	17	54.33	31.3
Cholorathional (2 g/l)	30.5	28.4	25	44.75	41.37	25.51	15	51.66	28.6
Copper oxychloride (2.5 g/l)	33.5	31.5	28	38.46	35.3	24.55	14	50.66	18
Control	39.99	40.6	45.25		28.78	19.43	10	33.66	3.3
C.D value	4.213				0.472	3.648	0.507	9.99	0.412
S.E (M) value	1.375				0.154	1.191	0.165	3.262	0.135
S.E (d) value	1.945				0.218	1.685	0.234	4.613	0.19
C.V value	9.364				0.669	7.646	1.800	11.078	5.031

scoring of early blight recorded very low in the plot sprayed with Azoxystrobin 18.2%+difenoconazole 11.4% SC (2.5 ml/l) and found to be superior than other treatments. Hence, field application of Azoxystrobin 18.2%+difenoconazole 11.4% SC could be recommended for use in tomato to control early blight disease with increased yield and no adverse effect on the crop. Similarly in experimental plots treated with biocontrol agents (*Trichoderma harzianum* and *Trichoderma viride*) showed less diseases incidence (61.10%) to 65.74%) with PDI (15.10 to 15.50%). Both of these isolates significantly enhanced plant growth parameter and fruit yield.

The results of the present investigation are comparable with Bartlett *et al.* (2002) who evaluated effect of strobilurin fungicides such as Azoxystrobin, Kresoxim methyl, Trifloxystrobin and Pyraclostrobin in influencing yield and quality of wheat, barley, greater yield benefits as compared to triazole based programmes. Tofoli *et al.* (2003) also evaluated the effectiveness of various groups of fungicides for controlling early blight and on yield of tomato and reported that the highest level of disease control, quality and Increase in fruit yields were obtained with Azoxystrobin followed by Mancozeb and Chlorothalonil. Sharma *et al.* (2018) found all fungicide treatments reduce the disease intensity as compared to untreated check. The lowest percent disease intensity (PDI) was observed in carbendazim 12 % + mancozeb 63 % WP @ 0.2 % (18.77) followed by difenoconazole 25 EC @ 0.025 % (20.59) and propiconazole 25 EC @ 0.025 % (21.52) treatments. Similarly, the highest yield of tomato fruits was recorded with carbendazim 12 % + mancozeb 63 % WP @ 0.2 % (35257 kg/ha) followed by propiconazole 25 EC @ 0.025 % (32328 kg/ha) and difenoconazole 25 EC @ 0.025 % (32202 kg/ha) when sprayed three times at an interval of 15 days starting from the initiation of the disease.

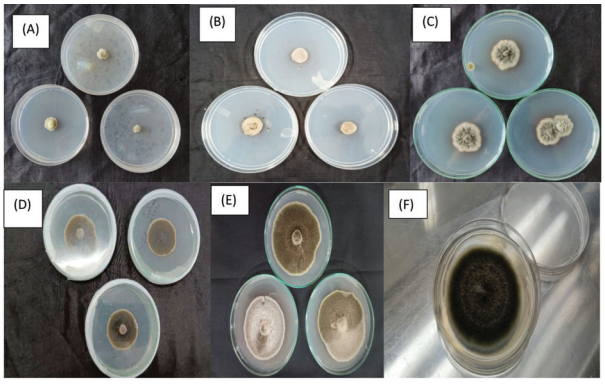

Plate 3. Efficacy of Azoxystrobin 18.2% + difenoconazole 11.4%SC (A), Propiconazole 25% EC(B), Mancozeb 75% WP (C), Chlorothalonil 75% WP (D), Copper oxychloride 50% WP (E) and control (Culture of *Alternaria solani*) (F).

Efficacy of biocontrol agents against *Alternaria solani* under field conditions

The biocontrol agents (*Trichoderma harzianum* and *T.viride*) found to be best effective in inhibiting the growth of *Alternaria solani* was found to be (PDI-65.74 to 61.10%) respectively. Therefore, both biocontrol agents significantly decreased the early blight disease incidence as compared to untreated control both under field condition and also both of these isolates significantly enhanced plant growth parameter and fruit yield. Similar type of results were also obtained by Kumar *et al*. (2008) foliar spray of *Trichoderma viride* (10^7 Cfu ml^{-1}) inoculated with the test fungus was found effective in reducing the disease severity under field conditions. Kumar *et al*., (2018) evaluated *Trichoderma harzianum* and *Trichoderma viride* against *Alternaria* under field conditions. Both biocontrol agents showed less disease incidence. Kulimushi *et al*. (2021) upon field application of *Trichoderma* against early blight which caused significant reduction in disease severity (%), which strongly supported the present results. As in the results, fruit yield after treatments with *T. harzianum* was higher in both seasons than the control and followed by *T. atroviride* and *T. longibrachiatum*.

CONCLUSION

Based on the above discussion it may be concluded that five fungicides and two biocontrol agents were evaluated following poison food technique and dual culture. At the highest concentration of fungicide, the least average mycelial growth of *Alternaria solani* was shown by Azoxystrobin 18.2%+difenoconazole 11.4% (20.01 mm) under *in vitro* conditions. Similar trend was also observed in field conditions. Among different fungicides, Azoxystrobuin+difenoconazole (15.5%) showed less disease severity as compared to other fungicides. Both biocontrol agents showing less diseases control percentage (61.10 to 65.74%) with diseases severity (15.5 to 17.6%) significantly enhanced plant growth parameter and fruit yield. Azoxystrobin 18.2%+difenoconazole 11.4% and bio control agents were favourable antagonist that can be used to control *A. solani*, helping in the management of early blight disease of tomato.

Declarations

There is no conflict of interest in the said study.

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