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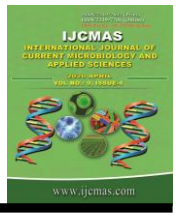

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Evaluation of Plant Extracts, Fungicides and Bio-agents against Early Blight Disease of Tomato Incited by *Alternaria solani* under *in vitro* and Field Conditions

Ravinder, Narender Singh*, Kushal Raj, Rakesh Kumar Chugh and Rakesh Sangwan

Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana- 125004, India

**Corresponding author*

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A B S T R A C T

Studies were undertaken to evaluate the plant extracts, fungicides and bio-agents against early blight disease of tomato caused by *Alternaria solani* under in vitro and vivo conditions. Among the four evaluated plant extracts against *A. solani*, neem leaf extract at 15% (w/v) was found to be most effective in inhibiting the mycelial growth of the pathogen with inhibition of 52.36 per cent, followed by combination of garlic clove and green chilli extract with the inhibition of 50.42 per cent. In case of evaluated fungicides, mancozeb 75WP at 0. 2% found to be most effective, followed by hexaconazole 4% and zineb 68% (0.2%) with the per cent inhibition of 72.55% and 67.17% respectively. *Trichoderma viride* was found to be most superior against *A. solani* with the maximum mycelial inhibition of 37.49% among the bio-agents. Among different treatments, viz., plant extracts, fungicides and bio-agents under field conditions mancozeb 75WP at concentration of 0.2% was found to be most effective in managing the early blight disease with least per cent disease intensity of 20.46 per cent and 83.28 per cent decrease in disease over the control. This reduction of disease was reflected in terms of yield i.e maximum yield was also recorded from the same treatment (18.5 K/plot) with 51.63 per cent increase in yield over the control plot.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and widely grown vegetables throughout the world. It ranks second in importance among the vegetable crops after potato. It is greatly affected by many biotic and abiotic stresses. Gleason and Edmunds (2006) reported that tomato crop suffers from many fungal diseases (Anthracnose, Early blight, Fusarium wilt, Late blight, Powdery mildew, damping-off (Pythium and Rhizoctonia) and fruit rot, bacterial diseases (Bacterial canker, Bacterial speck, Bacterial leaf spot, Bacterial wilt and Bacterial stem rot and fruit rot), viral diseases (Tomato leaf curl, Tomato mosaic and Tomato spotted wilt) and nematode diseases (Root-knot, Sting and Stubby-root).

Among the fungal diseases, early blight incited by *Alternaria solani* is one of the major serious concerns due to huge yield losses in tomato. It is classified in the Phylum Ascomycota, sub divison Pezizomycotina, class Dothideomycetes and order Pleosporales. The fungus usually infects tomato, potato and eggplant. The disease is favoured by warm temperature with extended periods of leaf wetness, dew, rainfall and dense plantation. The plants are more susceptible to this pathogen during fruiting period (Cerkauskas, 2005, Momel and Pemezny, 2006). Although the disease is termed as early blight, but it may occur at all stages of development. Early blight is a three-phases disease, which produce leaf spots, stem canker and fruit rot, but the foliar phase is more destructive which is responsible for significant economic losses sustained by tomato producer (Maiero and Barksdale, 1989). The early blight fungus can survive on the infected seeds for several days but it is still speculative whether the seed borne inoculum serve as a source of primary infection in the next season (Neergaard, 1945). The fungus over winters in infected plant debris, in or on the soil where it can survive at least one and perhaps several years.

Raja *et al.,* (2016) studied the management of the early blight of tomato by using the different plant extracts and reported that among the all used plant extracts neem leaf extract showed maximum inhibition (42.44%) over the *Alternaria solani* in all concentration (5, 10 and 15%), followed by the *Allium sativum* with the mean inhibition of 40.20 per cent. Wani *et al.,* (2017) evaluated twelve fungicides including systemic and non-systemic, five bioagents and eight plant extracts *in vitro* against *Exserohilum turcicum*, the causal agent of turcicum leaf blight of maize and reported that two foliar sprays with non-systemic fungicide, mancozeb 75 WP @ 0.25 per cent reduced the diseased intensity from 20.45 per cent in control to 5.69 per cent and increased the grain yield from 45.20 q/ ha in control to 52.50 q/ha; two foliar sprays with systemic fungicide, propiconazole 25 EC @ 0.1% reduced the diseased intensity to 6.11 per cent and increased the grain yield to 52.25 q/ha; two foliar sprays with plant extract, neem oil @ 5% reduced the diseased intensity to 10.90% and increased the grain yield to 49.90 q/ha while seed treatment with bioagent, *Trichoderma sp.* $2x10^8$ cfu/g @ 0.4% followed by two foliar sprays with mancozeb 75 WP @ 0.25% could further reduce the disease intensity to 5.40% and increase grain yield to 53.60 q/ha.

The control of early blight disease has been accomplished primarily by the application of chemical fungicides, long crop rotation, pasteurizing seedbed with steam or fumigants (Jones *et al.,* 1991) and efforts directed towards breeding resistant tomato cultivars. Very meagre information available on utilization of plant extracts, bio-agents and fungicides in managing the early blight disease of tomato. It is, therefore considered necessary to find out the remedial measures that could be cheap and eco-friendly for management of early blight of tomato.

Materials and Methods

In vitro evaluation of botanicals/plant extracts against early blight disease of tomato caused by *Alternaria solani*

The efficacy of four botanicals/plant extracts *viz.,* neem leaf extract, neem seed kernel extract, green chilli fruit extract and garlic clove + green chilli fruit extract @ concentrations of 5, 10 and 15 (w/v%) of each extract against *Alternaria solani* were tested *in vitro* using the standard procedure of poison food technique as given by Mayer (1962).

In vivo evaluation of botanicals/plant extracts for against *Alternaria solani* pathogen

To determine the efficacy of two effective plant extracts viz., neem leaf extract and garlic clove + green chilli fruit extract @ concentrations of 15 (w/v%) of each extract against the early blight of tomato, field trial were conducted at experimental area of the Department of Plant Pathology, CCS HAU, Hisar in *Rabi*, 2017-18 with a susceptible variety *i.e* Selection-7. Forty five days old seedlings were transplanted with a spacing of 50 cm × 50 cm. The plot size was 2.0 m × 2.0 m with three replications. Thirty days after transplanting, plants were inoculated with the spore suspension of *A. solani* having 1×10^6 spores per ml of sterilized water. The spore suspension was sprayed during evening hours for easy establishment of the pathogen. The plant extracts were sprayed after one day of spraying of spore suspension in their respective plot at 10 days interval between 30 to 50 days after transplanting. Only sterilized water was sprayed in control plots. Observations on disease severity were recorded at 10 days interval after spraying last plant extracts on the basis of 7 leaves randomly taken from each plot. Per cent disease intensity (PDI) was worked out by using 0 -5 scale (Mayee and Datar, 1986). The per cent disease control over check was worked out by following formula.

Per cent disease control =

$$\frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

In vitro evaluation of bio-agents against *A. solani*

The efficacy of two bio-agents namely, *Trichoderma viride* and *Pseudomonas fluorescence* @ concentrations of 0.1 , 0.5 and 2 (v/v) of each bio-agent was tested against *A. solani* by inhibition of mycelial growth on the PDA media using culture filtrate technique under *in vitro* condition.

In vivo evaluation of bio-agents against *A. solani*

To determine the efficacy of *T. viride* at 2 per cent concentration against early blight of tomato, the field trial was conducted at experimental area of the Department of Plant Pathology, CCS HAU, Hisar in *Rabi* season, 2017-18 with a susceptible variety (Selection-7). Forty five days old seedlings were transplanted with a spacing of 50 cm × 50 cm in Randomized Block Design. The plot size was 2.0 m × 2.0 m with three replications. Thirty days after transplanting, plants were inoculated with the spore suspension of *A. solani* having 1×10^6 spores per ml of sterilized water. The spore suspension was sprayed during evening hours for easy establishment of the pathogen. The culture filtrate of *T. viride* was sprayed after one day of spraying of spore suspension in their respective plots at 10 days interval between 30 to 50 days after transplanting and only sterilized water was sprayed in control plots. Observations on disease severity were recorded at 10 days interval after spraying last plant extracts on the basis of 7 leaves randomly taken from each plot. Per cent disease intensity was worked out by using 0 - 5 scale (Mayee and Datar, 1986). The per cent disease control over control was worked out by following formula.

Per cent disease control =

$$\frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

In vitro evaluation of fungicides

The efficacy of different fungicides viz., Mancozeb (75% WP), Propineb (70% WP), Propiconazole (25% EC) and Hexaconazole

+Zineb (72% WP) @ concentrations of 0.05 , 0.1 and 0.2 (w/v %) of each fungicides was evaluated against *A. solani* for inhibition of colony growth on the potato dextrose agar medium using poisoned food technique under *in vitro* conditions.

$$I = \frac{C-T}{C} \times 100$$

Where,
I = Per cent inhibition
C = Radial growth in control
T = Radial growth in treatment (fungicide)

In vivo evaluation of fungicides

To determine the efficacy of two effective fungicides against early blight disease, field trial was conducted at experimental area of the Department of Plant Pathology, CCS HAU, Hisar in *Rabi* (2017-18) with a susceptible variety *i.e.* Selection-7. Forty five days old seedlings were transplanted with a spacing of 50 cm × 50 cm. Thirty days after transplanting plants were inoculated with the spore suspension of *A. solani* having 1×10^6 spores per ml of sterilized water. The effective fungicides under in vitro conditions namely, Mancozeb (75% WP) @ 0.2% and Hexaconazole + Zineb (72% WP) @ 0.2% were sprayed after 24 hours of spraying of inoculum in respective plots at 10 days interval between 30 to 50 days after transplanting and observations on disease severity were recorded at 10 days interval after spraying last fungicide on the basis of 7 leaves randomly taken from each plot by using 0-5 scale (Mayee and Datar, 1986). The per cent disease control over control was calculated as

Per cent disease control =

$$\frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

The fruit yield in each plot was recorded separately. Increase in fruit yield over control was also calculated.

Results and Discussion

Evaluation of plant extracts, bio-agents and fungicides against *A. solani* under *in vitro* and field conditions

The efficacy of different treatments *viz.,* plant extracts, bio-agents and fungicides were evaluated under *in vitro* condition at their respective doses. Among the four evaluated plant extracts against *A. solani*, neem leaf extract at 15% w/v was found to be most effective in inhibiting the mycelial growth of the pathogen with inhibition of 52.36 per cent, followed by combination of garlic clove and green chili extract with the inhibition of 50.42 per cent. In case of evaluated fungicides, mancozeb 75WP at 0.2% found to be most effective, followed by hexaconazole 4% and zineb 68% (0.2%) with the per cent inhibition of 72.55% and 67.17% respectively. *Trichoderma viride* was found to be most superior against *A. solani* with the maximum mycelial inhibition of 37.49% among the bio-agents. (Table 1) Several workers have reported the efficacy of many plant extracts, fungicides and bioagents against the *A. solani* under *in vitro* conditions. Kamble *et al.,* (2000) tested six fungicides against *A. alternate*, under *in vitro* conditions. They reported that mancozeb was highly effective in inhibiting the mycelial growth followed by copper oxychloride and iprodine at 1000, 2000 and 3000 ppm.

Two plants extract (neem leaf extract and garlic clove and green chili extract), a bio-agent (*Trichoderma viride*) and two fungicides (mancozeb 75WP and hexaconazole 4% and zineb 68%), which were found most effective under *in vitro* were sprayed at their respective doses in the given schedule under the field conditions.

Table.1 Evaluation of plant extracts, fungicides and bio-agents against *Alternaria solani* under *in vitro* conditions

Treatment	Dose (%)	Per cent inhibition (%)	
T1	Neem leaf extract	5	42.88 (40.89)*
T2	Neem leaf extract	10	57.14 (49.09)
T3	Neem leaf extract	15	62.73 (52.36)
T4	Neem seed kernels extract	5	40.47 (39.49)
T5	Neem seed kernels extract	10	47.29 (43.43)
T6	Neem seed kernels extract	15	54.70 (47.68)
T7	Green chili fruit extract	5	37.42 (37.70)
T8	Green chili fruit extract	10	45.21 (42.23)
T9	Green chili fruit extract	15	51.98 (46.12)
T10	Garlic clove + Green chilli fruit extract	5	39.83 (39.11)
T11	Garlic clove + Green chilli fruit extract	10	52.45 (46.39)
T12	Garlic clove + Green chilli fruit extract	15	59.43 (50.42)
T13	mancozeb 75% WP	0.05	80.62 (63.87)
T14	mancozeb 75% WP	0.1	83.98 (66.45)
T15	mancozeb 75% WP	0.2	91.02 (72.55)
T16	propineb 70% WP	0.05	68.42 (55.79)
T17	propineb 70% WP	0.1	71.11 (57.47)
T18	propineb 70% WP	0.2	73.44 (58.96)
T19	propiconazole 25% EC	0.05	71.96 (58.02)
T20	propiconazole 25% EC	0.1	75.57 (60.36)
T21	propiconazole 25% EC	0.2	83.32 (65.89)
T22	hexaconazole +zineb (72% WP)	0.05	77.07 (61.38)
T23	hexaconazole +zineb (72% WP)	0.1	81.54 (64.56)
T24	hexaconazole +zineb (72% WP)	0.2	84.92 (67.17)
T25	*Trichoderma viride*	0.1	23.67 (29.09)
T26	*Trichoderma viride*	0.5	27.00 (31.29)
T27	*Trichoderma viride*	2	37.06 (37.49)
T28	*Pseudomonas fluorescence*	0.1	21.00 (27.24)
T29	*Pseudomonas fluorescence*	0.5	26.29 (30.83)
T30	*Pseudomonas fluorescence*	2	30.30 (33.38)
T31	Control	-	0.00
	C.D. at 5 %		2.07
	SE(m)		0.73

*= values in parentheses are angular transformed

Table.2 Evaluation of plant extracts, bio-agents and fungicides against early blight disease under field conditions

S.No.	Treatment	Dose (%)	*PDI (%)	PDOC (%)	*Yield (Kg/plot)	IYOC (%)
1.	Neem leaf extract	15	28.71 (32.34)*	61.34	16.8	37.70
2.	Garlic clove + Green chili fruit extract	15	32.42 (34.68)	56.35	15.5	27.04
3.	mancozeb 75% WP	0.2	12.43 (20.46)	83.28	18.5	51.63
4.	hexaconazole +zineb (72% WP)	0.2	19.86 (26.36)	74.62	16.5	35.24
5.	*Trichoderma viride*	2	48.57 (44.16)	34.61	15.8	29.50
6.	Control	-	74.28 (59.58)		12.2	
	C.D. SE(m)	1.20 0.38				

*Mean of three replications, *****values in parentheses are angular transformed, PDOC-per cent decrease in disease over control, IYOC-per cent increase in yield over control.

It was observed that among different treatments, mancozeb 75WP at concentration of 0.2% was found to be most effective in managing the early blight disease with least per cent disease intensity of 20.46 per cent and 83.28 per cent decrease in disease over the control. This reduction of disease was reflected in terms of yield *i.e.* maximum yield was also recorded from the same treatment (18.5 K/plot) with 51.63 per cent increase in yield over the control plot. However, *Trichoderma viride* was not found so effective in managing the early blight disease. Varma *et al.,* (2008) reported that foliar spray of *Trichoderma viride,* 24hours before challenge inoculation with the *Alternaria solani,* was found effective in reducing the disease severity under screen house conditions. They have also reported that effective management of early blight disease achieved with three sprays of mancozeb (2000ppm) under field condition (Table 2). Mancozeb (75% WP) and hexaconazole and zineb (72% WP) were found to be most effective among the fungicides under *in vitro* condition. Two plants extract (neem leaf extract and combination of garlic clove and green chili extract) and a bio-agent (*Trichoderma viride*) was also found effective in inhibiting the mycelial growth of *A. solani* among the plant extracts and bioagents, respectively. In field conditions, mancozeb 75WP was found to be most effective in reducing the early blight disease and increasing the fruits yield.

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