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Integrated Management of Early Blight of Tomato (*Lycopersicon esculentum* Mill.) caused by *Alternaria solani* (Ellis and Martin) Jones and Grout

Salimso Chapei, Narola Pongener* and Valenta Kangjam

Department of Plant Pathology, School of Agricultural Sciences and Rural Development, Nagaland University, Medziphema Campus-797 106, Nagaland, India

**Corresponding author*

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A B S T R A C T

The present study was carried out to find the integrated management of early blight of tomato (*Lycopersicon esculentum* Mill.) caused by *Alternaria solani.* Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop which is grown all over the world. Early blight of tomato, caused by *Alternaria solani* is one of the most important diseases in tomato. Two fungicides *i.e.* Mancozeb 75 WP and Hexaconazole 5 EC at different concentrations (0.1%, 0.2% and 0.3%), two bio-agents *viz.*, *Trichoderma harzianum* and *T. koningii* and two botanicals *i.e.* garlic bulb extract and neem leaves extract at different concentrations (5%, 10% and 15%) were evaluated *in vitro* against *A. solani*. On the basis of *in vitro* tests, both the fungicides caused significant inhibition of the mycelial growth. The highest inhibition of mycelial growth was recorded in all concentrations of Hexaconazole @ 0.1%, 0.2% and 0.3% with per cent inhibition of 94.44 followed by Mancozeb @ 0.2% with 93.33 per cent inhibition. *In vitro* screening of botanicals revealed that the per cent inhibition of mycelial growth was recorded most effective with 5% garlic bulb extract (84.81 %) followed by garlic @ 10% (79.63 %) and the least mycelial inhibition was exhibited by neem leaves extract@ 15% with 82.26%. Among the bioagents *T. harzianum* and *T. koningii* completely covered the mycelial growth of the test pathogen in 5 days and thus was highly antagonistic in nature and significantly reduced the growth of the pathogen. Under IDM, least mean value of disease severity was recorded from IDM_2 (soil application of *T. harzianum* + seedling dip with garlic extract + foliar spray with Hexaconazole @ 0.1%) with 1.98 per cent. This was followed by IDM_3 (burning with crop debris + seedling dip with Hexaconazole + foliar spray with garlic extract). Highest mean disease intensity of 23.04 per cent was recorded from IDM_0 (control). Maximum growth and yield was recorded from IDM_2 with 207.15 q/h with cost benefit ratio of 4.40.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the distinguished vegetable under *Solanaceae* family. It is one of the most popular vegetable crops grown all over the world. China leads in production and productivity of tomato followed by India,

United State of America, Turkey, Egypt and Iran. The total tomato cultivation area in India is 879.6 hectare and production is 18226.6 metric tons with the productivity of 20.7 metric tons per hectare. There has been a gradual increase in area under tomato while the production has been fluctuating due to various diseases and insect pest damage. There are several diseases on tomato caused by fungi, bacteria, viruses, nematodes and abiotic factors (Balanchard, 1992; Gomaa, 2001 and Abdel-Sayed, 2006). It is estimated that fungal diseases of tomato are responsible for 30% increase in production costs on fungicides used to combat these diseases (Grigolli *et al.,* 2011). Among the diseases, early blight, caused by *Alternaria solani* (Ellis and Martin) Jones and Grout, is one of the most important and frequent disease of the crop worldwide (Song *et al.,* 2011).

Alternaria blight is one the most important disease along with damping off, bacterial blight and tomato leaf curl virus (TLCV) in Varanasi with an average intensity varying from 35-40% every year. Losses may go up 80-86% when prolonged suitable weather condition prevails for early blight (Pandey and Pandey 2007). Early blight symptom appears as brown leaf spots marked with concentric rings to give a target effect. These spots enlarge slowly and may eventually destroy the leaves. With this in view, the present investigation has therefore been undertaken to study the usefulness of bio agents, botanicals, chemicals and cultural practices in the integrated management of early blight of tomato caused by *Alternaria solani*.

Materials and Methods

The infected leaf sample of tomato were collected from Central Institute of Horticulture Medziphema, Nagaland and the pathogen *Alternaria solani* was isolated from the naturally infected tomato plants showing typical symptoms of the disease. The infected portions of the leaves along with some healthy tissue were cut into small pieces. These pieces were surface sterilized with 0.1 per cent mercuric chloride solution for 30 seconds then washed thoroughly in sterile distilled water thrice to remove traces of mercuric chloride, if any, and then transferred aseptically to sterilized potato dextrose agar (PDA) plates.

They were incubated at 27±1° C and checked after every 24 hour for the growth of the fungus. The fungus was identified based on the morphological characteristics. Later, a bit of the fungal growth was transferred to PDA plates. The pure culture of the fungus was obtained by following hyphal tip culture under aseptic conditions (Rangaswamy, 1972).

The antagonists used in the present investigation, *viz., Trichoderma harzianum* and *T. koningii* were procured from the Biocontrol Laboratory, Department of Agriculture Medziphema, Nagaland. The selected botanicals neem leaves and garlic bulb were collected from Medziphema, Nagaland.

Preparation of plant extracts

Aqueous extracts of two plants were evaluated against *Alternaria solani*. Fresh leaves of neem and garlic bulb were collected and washed properly with distilled water. Hundred grams of fresh and washed plant materials (leaves, bulb) were ground well in a pestle and mortar with 100 ml (1:1 w/v) sterilized distilled water. The macerate was filtered through muslin cloth and the extract thus obtained was considered as standard extract (100 %) (Shekhawat and Prasada, 1971).

In vitro evaluation of fungicides on radial growth of pathogen

Mancozeb and Hexaconazole were tested at 0.1, 0.2 and 0.3 per cent concentrations for their efficacy on the mycelial growth of Alternaria *solani* by poison food technique (Nene and Thapliyal, 1982), to select the most promising chemicals for further evaluation under field conditions.

Required amount of chemicals were measured out and mixed in 100 ml molten PDA medium and 20 ml of poisoned media was poured into each of the sterilized petriplates (90 mm) under aseptic conditions. The fungal disc of the pathogen (5 mm) of 7 days old culture was cut out through the flame sterilized cork borer and transferred in the center of petriplates. Plates were incubated at 27±1°C. The observations were recorded after every 24 hours.

Effect of botanicals on radial growth of pathogen

The plant extract were screened under *in vitro* condition against *Alternaria solani* following poison food technique as suggested by Nene and Thapliyal, 1982, to select the most promising plants extract and for further evaluation under field conditions. Required volume of plants extract was measured out and mixed in sterilized molten PDA medium.

The medium was shaken thoroughly for the uniform mixing of the plant extracts. Twenty ml of poisoned media was poured into each of sterilized petriplates (90 mm) under aseptic conditions. Then 5 mm disc of 7 days old culture of the pathogen was cut out through flame sterilized cork borer and transferred in the center of petriplates. Plates were incubated at 27±1°C for 7 days. The observation was recorded after every 24 hours.

Effect of biocontrol agent on radial growth of pathogen

Biocontrol agents *viz., Trichoderma harzianum* and *Trichoderma koningii* were screened under *in vitro* condition against *Alternaria solani* following dual culture technique as suggested by Dennis and Webster, 1971, to select the most promising bio-control agent and further evaluation under field conditions for its antagonistic effect. Culture disc of 5mm diameter each of the fungal antagonists and the pathogen were cut with the help of a sterilized cork borer from the margin of the actively growing culture and transferred to PDA in petriplates (90 mm diameter) on opposite sides approximately at 10 mm from the periphery of the plate. A control having the test pathogen only was also kept for comparison. The petriplates were then incubated at 27±1° C till the control plates were comparatively covered fully by the pathogen.

The experiment was conducted in completely randomized block design (CRBD) with three replications in each treatment. Per cent inhibition of mycelial growth calculated using the following formula (Vincent, 1927).

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

Where, I= Per cent inhibition of mycelial growth
C= Colony diameter in control (mm)
T= Colony diameter treatment (mm)

In vivo evaluation

Based on the above *in vitro* test the best promising fungicide, botanical and bio agent were selected. The experiment was laid out in Randomized Block Design (RBD) with three replications. The treatment combinations were as follows.

IDM_0= Control

IDM_1= Mulching + seedling dip (*T. harzianum*) +foliar spray (garlic bulb extract)

IDM_2 = Soil application (*T. harzianum*) +seedling dip (garlic bulb extract) + foliar spray (Hexaconazole)

IDM_3= Burning with plant debris + seedling dip (Hexaconazole) + foliar spray (garlic bulb extract)

For recording of disease severity five plants were selected randomly from each plot at 15 days interval starting from the first appearance of the disease using a 0-9 scale (Mayee and Datar, 1986) which are rated as follows:

0: No infection
1: 1 – 10% leaves infected
3: 11 – 25% leaves infected
5: 26 – 50% leaves infected
7: 51 – 75% leaves infected
9: Above 75% leaves infected

Disease intensity was calculated with the above scales using the formula given by Davis *et al.,* (1993).

$$PDI = \frac{\text{Total disease ratings}}{(\text{total no.of leaves assessed} \times \text{maximum disease rating observed})} \times 100$$

Results and Discussion

Two selected fungicides *viz*., Mancozeb and Hexaconazole at different concentrations @ 0.1%, 0.2% and 0.3%, two botanicals extract *viz*., neem leaves and garlic bulb extract at 5%, 10% and 15% concentrations were evaluated using poison food technique.

Two bio- agents *viz., Trichoderma harzianum* and *Trichoderma koningii* were tested against the pathogen by dual culture technique. The average growth of pathogen was recorded at every 24 hours intervals. *In vitro* evaluation revealed that all the plant extract at different concentration were significantly effective in inhibiting the mycelial growth of *Alternaria solani.* However, the per cent inhibition of mycelial growth was recorded most effective with 5% garlic bulb extract (84.81 %). This was followed by garlic @ 10% with 79.63 per cent inhibition statistically at par with garlic @ 15% (78.52 %). The least mycelial inhibition was exhibited by neem leaves extract@ 15% with 64.81 %.

It was also observed that the test botanicals decreased the inhibition of mycelial growth with increase in their concentration. The results are in conformity with Chethana *et al.,* (2012) where extract of garlic (20%) was found to be most effective in causing 100 per cent inhibition of mycelial growth of *A. solani.* Narendrappa and Nandini (2013) also reported that the bio agent *Trichoderma harzianum,* bulb extract of *Allium sativum* and fungicide Avtar were the best in inhibiting the mycelial growth of *Alternaria solani.*

Among the botanicals tested, garlic bulb extract exhibited maximum per cent inhibition which may be due to presence of volatile oil which contains diallyl-disulphide, diallyl-tri-sulphide and sulphodoxides derived from *allium.*

The presence of antibiotic constituent in the form of phenolic, resinous, gummy and non-volatile substances of unknown nature in different botanicals which contribute to the inhibitory activity of the plant extracts has been reported by Skinner, 1955, Amonkar and Banerji, 1971 and Anonymous, 1972.

Two bioagents *viz., Trichoderma harzianum* and *Trichoderma koningii* were evaluated against the pathogen under *in vitro* condition (Table 1–4).

Table.1 *In vitro* evaluation of fungicides, botanicals and bio agents against mycelial growth of *Alternaria solani*

		24 hours		48 hours		72 hours		96 hours		120 hours		
		Mean radial growth (mm)	PI	Mean radial growth (mm)	PI	Mean radial growth (mm)	PI	Mean radial growth (mm)	PI	Mean radial growth(mm)	PI	Mean
T_0= Control		16.33	0.00	32.33	0.00	43.33	0.00	58.67	0.00	90.00	0.00	0.00
T_1=Mancozeb	0.1%	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	6.00	89.77 (71.35)	7.67	91.45 (73.04)	96.24 (78.82)
T_2=Mancozeb	0.2%	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	5.33	90.89 (72.46)	6.00	93.33 (75.07)	96.84 (79.76)
T_3=Mancozeb	0.3%	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	6.33	89.18 (70.82	8.67	90.37 (71.39)	95.91 (78.33)
T_4=Hexaconazole	0.1%	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	5.00	91.47 (73.02)	5.00	94.44 (76.37)	97.18 (80.33)
T_5=Hexaconazole	0.2%	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	5.00	91.47 (73.02)	5.00	94.44 (76.37)	97.18 (80.33)
T_6=Hexaconazole	0.3%	0.00	100.00 (90.00)	0.00	100.00 (90.00)	0.00	100.00 (90.00)	5.00	91.47 (73.02)	5.00	94.44 (76.37)	97.18 (80.33)
T_7=Neem	5%	9.33	43.10 (41.03)	11.33	64.67 (53.56)	14.00	67.58 (55.31)	19.33	67.03 (54.96)	23.67	73.70 (59.15)	63.21 (52.66)
T_8=Neem	10%	9.00	44.72 (41.95)	12.67	60.40 (51.04)	15.33	64.41 (53.41)	23.00	60.76 (51.23)	28.33	68.52 (55.87)	59.76 (50.63)
T_9=Neem	15%	12.00	26..48 (30.97)	14.00	56.25 (48.61)	19.00	56.02 (48.46)	25.67	56.21 (48.57)	31.67	64.81 (53.62)	51.95 (46.12)
T_{10}=Garlic	5%	0.00	100.00 (90.00)	6.00	81.30 (64.40)	6.33	85.35 (67.51)	10.33	82.33 (65.20)	13.67	84.81 (67.08)	86.75 (68.65)
T_{11}=Garlic	10%	0.00	100.00 (90.00)	5.33	83.27 (65.93)	7.33	83.03 (65.69)	10.33	82.35 (65.24)	18.33	79.63 (63.18)	85.65 (67.74)
T 1_2 = Garlic	15%	0.00	100.00 (90.00)	6.33	80.15 (63.51)	9.67	77.65 (61.80)	14.67	75.00 (60.01)	19.33	78.52 (62.40)	82.26 (65.09)
S. Em ±			**0.43**		**1.13**		**0.83**		**0.84**		**0.43**	
CD (p=0.05)			**1.25**		**3.29**		**2.40**		**2.44**		**1.25**	

Note: Figures in the table are mean values and those in parenthesis are angular transformed value

Table.2 *In vitro* evaluation of bio agents against *Alternaria solani*

Treatment	Inhibition percentage										
	24 hours		48 hours		72 hours		96 hours		120 hours		Mean
	Mean radial growth (mm)	PI	Mean radial growth (mm)	PI	Mean radial growth (mm)	PI	Mean radial growth (mm)	PI	Mean radial growth(mm)	PI	
T_1= *Trichoderma harzianum*	10.00	38.80	19.33	39.63	32.33	25.06	39.00	33.80	41.67	53.70	38.20
T_2 =*Trichoderma koningii*	9.00	44.95	21.67	32.58	34.33	20.44	41.33	29.48	43.67	51.48	35.79
t Stat		**-19**		**3.86**	**30.11**		**3.47**		**3.46**		
t Critical two-tail		**4.30**		**4.30**	**4.30**		**4.30**		**4.30**		

Table.3 Effect of integrated disease management on plant growth

Treatment	Plant height (cm)				Stem girth (cm)				Number of branches			
		Time				Time				Time		
	30DAT	60DAT	90DAT	Mean	30DAT	60DAT	90DAT	Mean	30DAT	60DAT	90DAT	Mean
IDM_0	14.47	36.67	37.33	**29.49**	1.44	1.96	2.45	**1.95**	2.87	4.27	6.43	**4.52**
IDM_1	15.60	42.20	43.53	**33.77**	1.48	2.23	2.53	**2.08**	3.33	5.67	6.33	**5.11**
IDM_2	17.40	49.60	50.93	**39.31**	1.81	3.05	3.20	**2.68**	5.00	7.67	8.33	**7.00**
IDM_3	16.80	46.73	47.60	**37.04**	1.69	2.24	2.65	**2.19**	3.67	6.00	7.00	**5.55**
Mean	**16.06**	**43.80**	**44.84**		**1.60**	**2.37**	**2.70**		**3.71**	**5.90**	**7.02**	

*Mean of three replication

SEm ± 0.85 CD (p=0.05) to compare treatment means over time =2.96 for plant height
SEm± 0.49 CD (p=0.05) to compare time means over treatment =1.20 for plant height
SEm± 0.80 CD (p=0.05) to compare interaction of treatment x time =2.41 for plant height
SEm ± 0.09 CD (p=0.05) to compare treatment means over time =0.33 for stem girth
SEm± 0.07 CD (p=0.05) to compare time means over treatment =0.21 for stem girth
SEm± 0.14 CD (p=0.05) to compare interaction of treatment x time =0.43 for stem girth
SEm ± 0.20 CD (p=0.05) to compare treatment means over time =0.71 for number of branches
SEm± 0.18 CD (p=0.05) to compare time means over treatment =0.55 for number of branches
SEm± 0.37 CD (p=0.05) to compare interaction of treatment x time =1.1 for number of branches

Table.4 Effect of integrated disease management on yield

Treatment	Yield			
	Number of fruit/plant	Weight of fruit/plant (gram)	Weight of fruit/plot (gram)	Yield (q/ha)
IDM_0	13.20	256.67	2860.67	71.30
IDM_1	16.10	390.00	6790.00	169.7
IDM_2	35.80	583.33	8286.67	207.15
IDM_3	20.13	460.00	7700.00	192.5
SEm±	1.10	2.22	547.51	
CD (p=0.05)	3.82	6.65	1894.64	

Table.5 Effect of integrated treatment on disease severity (per cent disease index)

Treatment	Time			
	45DAT	60DAT	75DAT	Mean
IDM_0	5.69 (13.80)	21.1 (27.34)	42.33 (40.58)	**23.04**
IDM_1	2.83 (9.68)	7.92 (16.34)	11.54 (19.85)	**7.43**
IDM_2	0.88 (5.38)	1.84 (7.79)	3.23 (10.35)	**1.98**
IDM_3	1.45 (6.91)	5.89 (14.04)	9.78 (18.22)	**5.70**
Mean	**2.71**	**9.18**	**16.72**	

* Mean of three replication
SEm ± 0.96 CD (p=0.05) to compare treatment means over time =3.35
SEm± 0.45 CD (p=0.05) to compare time means over treatment =1.36
SEm± 0.91 CD (p=0.05) to compare interaction of treatment x time =2.73

The results of the experiment were subjected to paired-t test at 5 % level of significance, which revealed that effect of *T. harzianum* bio agents was significantly better than *T. koningii* in reducing the growth of the pathogen. Shuakkat and Rao (2013) also reported that culture filtrate of all the five *Trichoderma* species (*viz., T. viride, T. virens, T. harzianum, T. koningii* and *T. pseudo koningii*) retarded the growth of *A. solani* but *T. viride* and *T. harzianum* more strongly suppressed the growth of *Alternaria solani*. The inhibition of *Alternaria solani* in the presence of *Trichoderma* spp could be due to antibiosis or hyperparasitism. Both *T. harzianum and T koningii* overgrew on the pathogen colony and complete invasion and sporulation occurred after five days.

Effect of integrated disease management on disease severity of early blight of tomato

Under IDM there were three treatments where different individual methods were integrated together and applied for the experiment *viz.*, IDM_1 (mulching with polyethylene sheet + seedling dip with Hexaconazole + foliar spray with garlic extract), IDM_2 (soil application of *T. harzianum* + seedling dip with garlic extract + foliar spray with Hexaconazole) and IDM_3 (burning with crop debris + seedling dip with Hexaconazole + foliar spray with garlic extract) depicts the disease severity with respect to integrated disease management and dates of observation (45, 60 and 75 DAT) against the early blight of tomato (Table 5).

The experimental data were subjected to measurement over time analysis, and it showed that effect of three IDMs on disease severity of early blight of tomato were significant. The effect of time as well as interaction effect of IDMs and time was found significant at 5% level of significance. Minimum disease intensity was recorded from IDM_2 with 1.98 %. This was followed by IDM_3 (5.70 %) which was statistically at par with IDM_1.

There were also significant variations on disease intensity at different time of observation over treatments. The highest mean value of 16.72 was recorded at 75 days after transplanting. The interaction between the treatments and time of observation revealed significant variations where IDM_2 gave the lowest disease intensity of 1.98 per cent and the highest was recorded from IDM_0 with 42.33 per cent.

All the treatments were significantly superior over control in reducing the disease severity. Integrated management of early blight of tomato not only checked the disease severity but also increased the yield of tomato fruits. The better performance of the integrated methods over control may be due to the combined action of the fungicide, botanical, bio agent and cultural practices. The present findings are in conformity with the observations recorded by Rashmi and Vishunavat (2012) who was of the view that cultural practices when it is integrated with fungicides reduced the per cent disease index and increased the yield.

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