

# Investigations on Variability and Eco-friendly Management of *Alternaria alternata* Causing Tomato Blight

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Different isolates of *Alternaria alternata* showed variations in symptoms, growth characteristics, colony diameter, pattern of sporulation, size of conidia and mean number of septa. Of four isolates, Aa-1 produced maximum colony diameter (62.00 mm), sporulation, percent infection (80.00), conidia length (69.75 $\mu$ m), beak length (24.45 $\mu$ m) and horizontal septation (8.45). The shape and colour of the culture of different isolates varied from compact colony growth, dark brown colour in the center having white periphery, concentric rings prominent in AA-1 isolates. Out of six fungicides, Mancozeb was found to be the most effective against mycelial growth of *A. alternata*. In respect to plant extract and bio-control *Azadirachta indica* and *Trichoderma viride* caused maximum inhibition of *A. alternata* growth as compared to other bio-agents. In pot and field experiments, maximum efficacy of per cent disease control (66.56) and yield (22.99 t/ha) was recorded in seed treatment and two foliar spray of Mancozeb.

**Keywords:** *Alternaria alternata*, tomato blight, morphological variability, plant extract, bio-agent, fungicides

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## INTRODUCTION

Importance of vegetables in human nutrition is well established besides cereals and milk. Tomato (*Lycopersicon esculentum* Mill.) is one of the important vegetable crops which are consumed either raw or cooked. It is a rich source of minerals and vitamins (Choudhary 1996). Several factors have been identified that are responsible for dwindling yield of tomato, which most importantly include poor quality seeds, incidence of diseases and pests and adverse climatic conditions. Among the fungal diseases, leaf blight incited by *Alternaria alternata* is a serious constraints in the production of tomato. This diseases becomes more severe in June -July sown crops than the winter crop resulting into losses as high as 78 per cent (Singh 1998). The pathogen was first described by Ellis and Martin, (1882) from the leaves of potato collected from the New Jersey. Rands (1917) was the first to prove

that the same pathogen incited the leaf and stem disease on both tomato and potato. In India this disease was first noticed by Butler in Faridabad, U.P in 1905 (Ghosh 1972).

## MATERIALS AND METHODS

### Culture and morphological characters of the isolates

The fungal pathogen collected from infected plant parts were grown on PDA. The individual isolates of *Alternaria alternata*, removed from the periphery of 7-days old culture were aseptically placed in the centre of the PDA plate, keeping four replications of each isolate. The plates were incubated at 25 $\pm$ 2 $^{\circ}$ C and variations in growth pattern and colony growth (diameter) of fungus in all isolates were recorded. Spore production by each isolate was determined by removing agar-plugs (5 mm diameter) from three linear spots across the centre of the colony, which were suspended in 10 ml ster-

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ile water in glass test tubes and were agitated twice for about 10 seconds in a vortex shaker to dislodge conidia. The number of conidia in the resultant suspension was determined using haemocytometer, and was expressed as number of conidia per mm<sup>2</sup> of medium. Spore size (length and width) measurements were taken by measuring 20 spores of each isolate using stage and ocular micrometer and septa were counted in 20 spores from each isolate. Conidial development and variation in conidial morphology of isolates were studied by slide culture method.

### Efficacy of fungicide, bio-agents and botanicals on *A. alternata* (in vitro)

**Fungicides :** The effect of different concentrations viz 100, 250, 500 and 1000 a.i. ppm of sex fungicides was evaluated *in vitro* against *A. alternata* using poisoned food technique (Nene and Thapliyal 1979). The calculated fungicides in proper concentrations were thoroughly mixed in the sterilized PDA before pouring into Petri plates so as to get the desired concentration of each fungicide separately. Twenty ml of fungicide amended medium was poured aseptically in each 90 mm sterilized Petri plate and allowed to solidify and medium without fungicides served as control. The plates were inoculated with 5 mm disc of 7 days-old culture of *A. alternata*. The inoculated plates were incubated at 25 ± 2 °C in an incubator. The experiments were conducted in completely randomized block design with four replications in each treatment. After 7 days of inoculation when the control plates were full with the fungal growth, the diameter of the colony

in each plate was measured. Per cent inhibition of growth of *A. alternata* was calculated using the formula given by Bliss (1934).

**Bio-agents :** Dual culture method was used for assessing inhibition of radial growth of the pathogen by the antagonists. The antagonists and the individual pathogen were inoculated simultaneously 5 cm apart on 0.1 per cent filtered malt extract agar in Petri plates. The control plates were inoculated by only bits of same pathogen, four replications were maintained for each combination. The plates were incubated at 25 ± 2°C. The measurement of radial growth of the pathogen was recorded in each plate after 7 days. The per cent inhibition of growth was calculated as per the formula given by Bliss (1934).

**Botanicals :** Fresh plant leaves were first collected and washed, surface sterilized (2 % sodium hypochlorite), followed by 3 washings with sterile distilled water and subsequent sterilized coverage for air drying. The plant materials were weighed, crushed with 80 % ethanol (1 : 1 w/v) in waring blender. The mixture was filtered through two fold muslin cloth and the filtrate was evaporated and clear extract was diluted with sterile distilled water to make volume of 1 : 1 (w/v). This was considered as 100 per cent concentration which was used for experimental work (Sharma et al. 2003). For evaluation of antifungal activities of plant extracts on mycelial growth of fungi, desired concentrations (10, 20, 30 and 40%) were obtained by adding appropriate amount of standard solution of plant extracts to PDA medium. Each plate was

**Table 1. Colony morphology and sporulation of *Alternaria alternata* isolates grown on PDA**

Isolates	Colony diameter (mm in 7d)*	Sporulation**	Colony characteristics
AA-1	62.00	++++	Compact colony growth, dark coloured in the center having white periphery, concentric rings prominent.
AA-2	58.20	++	Light brown to dark black colonies, concentric rings are depictable.
AA-3	58.60	+	Loose spreading type growth, concentric rings not clear.
AA-4	60.20	+++	Cottony growth, dark in color in center gradually and becoming lighter towards periphery, concentric rings prominent.
SEm±	1.64		
CD (0.05)	4.88		

\*Average of five replication; \*\* Sporulation categories: + scanty; ++ moderate; +++ good; ++++ abundant

**Table 2. Conidial\* morphology of different isolates of *Alternaria alternata***

Isolate	Length (µm)	Breadth (µm)	Septation		Beak length(µm)	Beak septation
			H	V		
AA-1	69.75±1.87	15.30±0.44	8.45±0.42	2.30±0.23	25.45±2.91	2.25±0.29
AA-2	54.90±1.83	16.05±0.41	7.85±0.30	2.15±0.21	18.50±2.89	4.45±0.36
AA-3	59.30±1.62	15.85±0.38	7.35±0.27	2.40±0.23	21.25±2.76	3.00±0.47
AA-4	61.50±1.59	14.60±0.54	7.70±0.30	2.05±0.20	22.40±2.62	3.45±0.47

\*Average of 20 observations with standard error of mean (±).

inoculated with 5 mm diameter mycelial disc of pathogen taken from 7 days old culture. The inoculated plates were incubated at  $25 \pm 2$  °C for 7 days and diameter of colony was measured in each case and per cent inhibition of growth were calculated as per the formula of Bliss (1934).

#### Evaluation of fungicides, bio-agents and botanicals for tomato blight Under pot house condition

The antifungal effects of plant extracts, bio-agents and fungicides were assessed against *Alternaria alternata* in pots. Plant extracts viz., neem (*Azadirachta indica*), datura (*Datura stramonium*), jatropha (*Jatropha curcas*), karanj (*Cesalipina badalleliac*), biological agent viz., *Trichoderma viride*, *T. harzianum*, two strains of *Pseudomonas* spp. and fungicides viz., carbendazim, thiram, iprodione, vitavax, topsin M and mancozeb were used as seed treatment and foliar sprays. The experiment was conducted in pots in completely randomized block design with three replications. For comparison, inoculated control plants were maintained without application of any treatment. Since small quantities of seeds were to be treated, these were soaked in solutions of plant extracts, biological agents and fungicides and in sterilized distilled water as control for one hour. The treated seeds were allowed to air dry and then sown in the pots. After germination of seeds, thinning of seedling was done to maintain 5 seedlings per pot. Observations for blight were recorded on individual plants after 30 days of inoculation, using the 0-5 disease rating scale.

#### Under field condition

On the basis of *in vitro* and pot experiment performance and the antifungal effects of plant extract, bio-agents and fungicides, evaluation was done to observe suppression

of tomato blight in the field. The experiment was conducted in 3.6 x 1.8 m plots, keeping three replications for each treatment along with control in a randomized block design. Plant extracts, neem (*Azadirachta indica*) and datura (*Datura stramonium*), bio-agents, *Trichoderma viride* and *T. harzianum* and fungicides carbendazim, thiram, iprodione, vitavax, topsin M and mancozeb were used both for seed treatments and foliar sprays. The seeds treated with different treatments were air dried and sown in the nursery. Inoculations were done after 30 days of sowing by spray inoculation of conidial suspension. Two foliar sprays of different treatments were applied at 21 days interval. The inoculated control plots were maintained without application of any treatments. Observations for blight disease were recorded after 30 days of spraying of plant extracts, bio-agents and fungicide formulations on 0-5 disease rating scale and PDI was calculated. The per cent efficacy of disease control (PEDC) was determined and fruit yield was also recorded for each treatment.

## RESULTS AND DISCUSSION

During the present investigations four isolates of *Alternaria alternata* were collected from different locations of Udaipur district, Rajasthan, India showed variations in colony diameter, rate of sporulation, shape and colour of the colony. Maximum mean colony diameter 62.00 mm was recorded in isolate AA-1. The shape and colour of the culture of different isolates varied from compact colony growth, dark brown coloured in the center having white periphery, concentric rings prominent in AA-1 isolates (Table-1). *Alternaria solani* isolate differed from all other isolates and recorded the highest

**Table 3. *In vitro* efficacy of different fungicides against mycelial growth of *Alternaria alternata***

Fungicides	Colony diameter (mm)				Per cent growth inhibition			
	Concentration (ppm)				Concentration (ppm)			
	100	250	500	1000	100	250	500	1000
Carbendazim	69.33	58.00	29.67	4.33	22.95 (28.63)	35.55 (36.60)	67.04 (54.97)	95.20 (77.34)
Mancozeb	11.00	6.67	4.33	0.00	87.79 (69.55)	92.63 (74.24)	95.20 (77.34)	100.00 (90.00)
Iprodione	16.00	13.00	9.00	0.00	82.23 (65.07)	85.57 (67.67)	90.02 (71.58)	100.00 (90.00)
Thiram	24.00	16.33	12.33	0.00	73.34 (58.91)	81.86 (64.79)	86.30 (68.28)	100.00 (90.00)
Vitavax	49.00	40.00	38.00	17.00	45.55 (42.45)	55.56 (48.19)	57.78 (49.48)	81.12 (64.25)
Topsin-M	24.33	20.00	14.33	12.00	72.96 (58.67)	77.78 (61.88)	84.08 (66.48)	86.68 (68.59)
Control	90.00	90.00	90.00	90.00	0.00	0.00	0.00	0.00
					CD(0.05)	CD(0.05)	SEm±	CD (0.05)
Fungicide					0.294	0.836	0.245	0.696
Concentration					0.589	1.672	0.490	1.392

\* Average of three replications \*\* Figures in parentheses are angular transformed value

**Table 4.** *In vitro* efficacy of different botanicals on growth of *Alternaria alternata*

Botanicals	Colony diameter (mm)				Per cent growth inhibition (%)			
	Concentration (%)				Concentration (%)			
	10	20	30	40	10	20	30	40
<i>Azadirachta indica</i>	24.33	14.67	2.33	0.00	72.96 (58.67)	83.70 (66.19)	97.42 (80.76)	100.00 (90.00)
<i>Datura stramonium</i>	60.00	24.00	16.00	6.00	33.32 (35.25)	73.33 (58.91)	82.23 (65.07)	93.36 (75.07)
<i>Cesepina badalleiac</i>	61.67	34.00	17.00	9.00	31.47 (34.12)	62.22 (52.07)	81.12 (64.24)	90.02 (71.58)
<i>Jatropha curcas</i>	66.33	37.67	21.00	9.33	26.28 (30.84)	58.15 (49.69)	76.67 (61.12)	89.65 (71.23)
Control	90.00	90.00	90.00	90.00	0.00	0.00	0.00	0.00
					SEm±	CD (0.05)	SEm±	CD (0.05)
Botanicals					0.338	0.972	0.280	0.807
Concentration					0.676	1.944	0.561	1.615

Average of three replications \*\* Figures in parentheses are angular transformed values

**Table 5.** *In vitro* testing of bio-control agents against *Alternaria alternata* through dual culture technique

Bio control agents	<i>Alternaria alternata</i>	
	Linear growth (mm)*	Per cent inhibition of growth*
<i>Trichoderma viride</i>	15.75	82.53(65.29)
<i>Trichoderma harzianum</i>	18.50	79.45(63.05)
<i>Pseudomonas fluorescence RRLJ-01</i>	33.25	63.06(52.57)
<i>Pseudomonas fluorescence RRLJ-01</i>	37.50	58.33(49.80)
Control	90.00	0.00
SEm ±	0.652	1.614
CD (0.05)	1.965	5.023

\*Average of four replications; the values in the parentheses are angular transformed

mean radial growth 60 mm as compared to other isolates (Babu *et al.* 2000a). Studies on morphological characteristic of four isolates of *A. alternata* showed significant variations in conidial morphology. The average conidial length was maximum in isolate AA-1 (69.75 ± 1.87 mm), while conidial breadth was maximum in the isolate AA-2 (16.05 ± 0.41 mm). The maximum number of horizontal septa were associated with the isolate AA-1 (8.45 ± 0.42) and least septation was observed in isolate AA-3 (7.35 ± 0.27). The number of vertical septa was higher in the isolate AA-3 (2.40 ± 0.23) and least number of vertical septa was noticed in the isolate AA-4

(2.05 ± 0.20) (Table-2). Varma *et al.* (2007) also observed that isolates of *A. solani* shown variation with respect to conidial size, beak length and septation. The average conidial length was maximum in isolate YAS (224.9 ± 1.63 mm) followed by isolate SAS (175.6 ± 1.72 mm) and least septation was observed in isolate DLAS (5.0 ± 0.19). The number of vertical septa was higher in the isolate HAS-II (2.2 ± 0.13). Tetarwal *et al.*, (2008) also studied the morphological and pathogenic variability of *A. alternata* and conidial length and width was found between 37.55 to 51.60 mm and 13.60 to

**Table 6. Efficacy of different treatments on per cent disease incidence and per cent efficacy against tomato diseases in pot experiment**

Treatments	Concentration	<i>Alternaria alternata</i>	
		PDI	PEDC
Carbendazim	500 ppm	41.76(40.26)	51.24(45.71)
Mancozeb	500 ppm	20.63(27.01)	75.92(60.61)
Iprodione	500 ppm	21.49(27.62)	74.93(59.96)
Thiram	500 ppm	27.22(31.45)	68.20(55.67)
Topsin-M	500 ppm	34.10(35.73)	60.21(50.89)
Vitavax	500 ppm	37.57(37.80)	56.15(48.53)
<i>Azadirachta indica</i>	40%	32.51(34.76)	62.04(51.97)
<i>Datura stramonium</i>	40%	34.90(36.21)	59.34(50.38)
<i>Cesalipina badalleliac</i>	40%	39.84(39.14)	53.45(46.98)
<i>Jatropha curcas</i>	40%	42.56(40.72)	50.30(45.17)
<i>Trichoderma viride</i>	-	32.24(34.60)	62.37(52.16)
<i>Trichoderma harzianum</i>	-	34.11(35.73)	60.23(50.90)
<i>Pseudomonas fluorescence</i> RRLJ-01	-	41.01(39.82)	52.15(46.23)
<i>Pseudomonas fluorescence</i> RRLJ-02	-	43.57(41.31)	49.19(44.53)
Inoculated control	-	85.71(67.79)	0.00
SEm ± CD (0.05)		0.351 1.013	0.377 1.089

Average of three replications \*\* Figures in parentheses are arcsine angular transformed value

**Table 7. Efficacy of different treatments on per cent disease incidence and per cent efficacy against *Alternaria alternata* in the field experiment**

Treatments	Concentration (%/ppm)	Per cent disease incidence			Per cent efficacy of disease control		
		2007	2008	Mean	2007	2008	Mean
Carbendazim	500 ppm	45.31(42.31)	43.03(40.99)	44.17	38.74(38.49)	42.26(40.55)	40.50
Mancozeb	500 ppm	25.36(30.24)	24.23(29.49)	24.79	65.60(54.09)	67.52(55.26)	66.56
Iprodione	500 ppm	26.10(30.72)	24.89(29.92)	25.49	64.63(53.51)	66.51(54.64)	65.57
Thiram	500 ppm	29.85(33.12)	27.83(31.84)	28.84	59.57(50.52)	62.77(52.40)	61.17
Topsin-M	500 ppm	38.23(38.19)	35.55(36.60)	36.89	48.34(44.05)	52.57(46.47)	50.45
<i>Azadirachta indica</i>	40%	37.15(37.55)	35.64(36.65)	36.39	49.68(44.82)	52.30(46.32)	51.12
<i>Datura stramonium</i>	40%	40.21(39.36)	38.59(38.40)	39.40	45.51(42.42)	48.37(44.07)	46.94
<i>Trichoderma viride</i>	-	30.97(33.82)	29.88(33.14)	30.42	58.17(49.70)	60.00(50.77)	59.08
<i>Trichoderma harzianum</i>	-	31.84(34.35)	30.51(33.53)	31.17	56.98(49.01)	59.08(50.23)	58.03
Inoculated control	-	73.97(59.32)	74.75(59.84)	74.36	0.00	0.00	
SEm ± CD (0.05)		0.68 2.01	0.87 2.58	-	0.51 1.51	0.64 1.81	

\* Average of three replications \*\* Figures in parentheses are angular transformed values

**Table 8. Effect of different treatments on tomato yield after inoculation with *Alternaria alternata* in the field experiment**

Treatments	Concentration (%/ppm)	Tomato yield(t/ha)against <i>Alternaria alternata</i>		
		2007	2008	Mean
Carbendazim	500 ppm	16.84(24.23)	18.36(25.37)	17.60
Mancozeb	500 ppm	22.86(28.57)	23.13(28.74)	22.99
iprodione	500 ppm	21.90(27.90)	22.71(28.46)	22.30
Thiram	500 ppm	20.20(26.71)	20.79(27.12)	20.49
Topsin-M	500 ppm	18.38(25.38)	19.68(26.34)	19.03
<i>Azadirachta indica</i>	40%	19.20(25.99)	20.50(26.92)	19.85
<i>Datura stramonium</i>	40%	18.78(25.68)	19.89(26.49)	19.33
<i>Trichoderma viride</i>	-	20.05(26.60)	22.57(28.37)	21.31
<i>Trichoderma harzianum</i>	-	20.51(26.93)	21.13(27.37)	20.82
Inoculated control	-	15.81(23.43)	14.16(22.11)	14.98
SEm ±		0.41	0.33	
CD (0.05)		1.22	0.98	

\* Average of three replications \*\* Figures in parentheses are angular transformed value

100 and 30 mm respectively. Number of horizontal and vertical septa varied between 4 to 8 and 2 to 4 respectively.

In present study out of six fungicides Mancozeb was found to be the most effective against mycelial growth of *A. alternata* and gave 87.79, 92.63, 95.20, 100% growth inhibition in all tested (100, 250, 500 and 1000 ppm) concentrations. Under pot house condition results revealed that there was clear reduction in the disease severity when fungicides were applied as seed treatment and foliar sprays. Mancozeb (500 ppm) gave maximum per cent efficacy 75.92 per cent of blight diseases, where as Iprodione (500 ppm) and Thiram (500 ppm) also show effectiveness resulting in per cent efficacy in disease control as 74.93 and 68.20 per cent against blight infection. Under field condition mean data of two years revealed that maximum per cent efficacy of disease control 66.56 percent were observed in seed treatment followed by two foliar sprays of Mancozeb. In this treatment 24.79 mean PDI was also observed as compared to 74.36 mean PDI in the control. It was also observed that all the treatment increase the fruit yield as against control. Mean data of two year revealed that maximum fruit yield (22.99 t/ha) were observed in seed treatment and two foliar spray of Mancozeb as the compared to (14.98 t/ha) in the control. Vishwakarma (1989) found that Mancozeb gave minimum disease index 34.75 as against control 63.64 and significant higher yields were achieved in the plots sprayed with Mancozeb and increased yield over check by 44.48 per cent against early blight of tomato. Babu *et al.*, (2000b) reported that spraying with mancozeb caused the maximum disease reduction 77.27 per cent in pot experiment and lowest percent disease incidence 13.33 per cent in the field and resulted in the highest yield among all treatments against tomato leaf blight. Deora *et al.* (2004) found that mancozeb showed the best efficacy in controlling the blight disease of tomato, with more than 50 per cent disease control compared to control. Kanzaria *et al.* (2005) reported that sig-

nificant minimum early blight disease intensity as 26.50 per cent and highest fruit yield (23,833 kg/ha) was recorded in the combinations of both nursery management and spray of mancozeb at 0.3 per cent, which was also found to be economical. Kumari *et al.* (2006) reported that Mancozeb was found to be the most effective fungicide in checking the mycelial growth of *A. alternata*.

In present investigations out of four plants extracts *Azadirachta indica* caused maximum inhibition 100 percent of *A. alternata* mycelial growth followed by *Datura stramonium* 93.36 percent at 40 per cent concentration. Botanicals particularly *Azadirachta indica* and its kernels have shown good fungicidal potentials against several pathogenic fungi including *Rhizoctonia solani*, *Macrophomina* sp., *Fusarium* sp. *Alternaria* sp. *Aspergillus* sp. (Marriappan 1998). Hassanein *et al.*, (2008) tested leaf extract of aqueous ethanol and ethyl acetate of neem at 5, 10, 15 and 20% concentration and reported that with neem, the inhibition percentages were 17.88, 23.66, 52.77 and 70.55% for *Alternaria solani* at four used concentration..

The bio-control agents, *T. viride* was found most effective causing significant suppression 82.53 per cent of mycelial growth followed by *T. harzianum* which caused 79.45 per cent inhibition of growth as compared to other bio agents against *A. alternata* diseases respectively. Many scientists also supported the results of bio control against *A. alternata*. *T. harzianum* and *T. viride* were significantly effective inhibiting the mycelial growth of *Alternaria solani*, *in vitro* (Babu *et al.*, 2000b). Hafeez *et al.*, (2001) found that *T. harzianum* were effective *in vitro* retarding the mycelial growth of *Alternaria solani*. Farnawany (2006) observed that seed or soil treatment with *T. harzianum* significantly reduced the disease incidence of *Alternaria* blight of tomato. Reduction of infection percentage was more pronounced in seed than in soil treatment and was up to 60 per cent compare to control. Akba-

ri and Parakhia (2007) tested the antagonistic properties of *Trichoderma* spp. *Bacillus* isolates and *Streptomyces* spp. against *A.alternata* and reported the *T. viride*-I, *T. harzianum*-IV and V and *Bacillus subtilis* G. showed strong antagonism as 79 percent inhibition of *A. alternata*. Deepak *et al.*, (2008) observed the maximum inhibition 85.45 per cent of the mycelial growth of *Alternaria burnsii* in the presence of *T. harzianum strain II* where as minimum blight 36.15 PDI was observed when *T. harzianum strain II* applied to the soil at the rate of 24 g/m<sup>2</sup>.

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