

Management of *Alternaria* blight (*Alternaria alternata*) of tomato through novel combined formulations of fungicides

RATAN LAL SHARMA^{1*}, R. R. AHIR¹, ASTHA SHARMA¹, PINKI SHARMA² AND
PURUSHOTAM SHARMA³

¹Department of Plant Pathology, Agricultural Research Station, (Agriculture University, Jodhpur), Keshwana, Jalore - 343001, Rajasthan,

²Department of Plant Pathology, and ³Department of Entomology, S.K.N. College of Agriculture (SKNAU), Jobner, Jaipur - 303329, Rajasthan

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The present investigation aimed to find the most efficient and novel combined formulation of fungicides belonging to triazole and strobilurin groups for managing *Alternaria* blight (*Alternaria alternata*) of tomato under open field conditions. Efficacy of seven fungicides like propineb, hexaconazole, propiconazole, azoxystrobin, mancozeb, tebuconazole+trifloxystrobin and carbendazim + mancozeb was evaluated *in vitro* and *in vivo*. *In vitro* study was carried out through poisoned food technique, while field experiment was conducted for two consecutive years (2017 and 2018) at Jobner (Jaipur), India. Two foliar sprays were applied at 45 and 60 days after transplanting (DAT), and disease intensity was recorded at 75 DAT. Hexaconazole (@ 300 and 500 ppm) completely inhibited mycelial growth, followed by tebuconazole + trifloxystrobin (@ 500 ppm). In field conditions, two foliar sprays of hexaconazole (@ 0.2%) at 15 days intervals provided maximum disease reduction (79.74%) with increased fruit yield (88.51%) over control, followed by tebuconazole + trifloxystrobin (76.33% and 82.35%, respectively). *Alternaria* blight is one of the significant biotic stresses that limit crop productivity and grower's prosperity. Systemic fungicides, particularly triazoles, play a substantial role in preventing infection by *Alternaria* sp. Thus, two foliar sprays of hexaconazole (0.2%) at 15 days intervals reduced disease intensity effectively with increased fruit yield and profitability.

Keywords: *Alternaria* blight, fungicide combinations, tomato

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) has the first position among processing crops in India, and it is the world's second most important consumed vegetable crop after potato (Kumar, 2015).

Its fruits are famed for their attractiveness, nutritional value, and various medicinal uses. It is cultivated throughout the world and is a rich resource of lycopene, β -carotene, β -tocopherol and mineral nutrients (Meena *et al.* 2018).

In India, the tomato crop is mainly cultivated in Odisha, Andhra Pradesh, Madhya Pradesh, Karnataka, West Bengal, Chhattisgarh,

Telangana, Bihar, Gujarat, Rajasthan and Uttar Pradesh. In Rajasthan, it is mainly grown in Jaipur, Dausa, Alwar, Tonk, Dholpur, Bharatpur and Chittorgarh districts with an area of 85.00 million hectares with an annual production of 21.00 million tonnes.

The tomato crop is susceptible to abiotic stresses like adverse temperature, salinity, drought, moisture, environmental pollution, and biotic stresses, including insect pests and diseases from emergence to harvest. The tomato crop is attacked by many diseases caused by fungi, bacteria, viruses, nematodes etc., in many countries (Abada *et al.* 2008). Among diseases caused by fungi and fungi-like organisms, *Alternaria* blight (*Alternaria* sp.), late blight (*Phytophthora infestans*), Septoria leaf blight (*Septoria lycopersici*), powdery mildew (*Oidiopsis*

*Correspondence : sharmaratanlal851@gmail.com

taurica), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), collar rot (*Sclerotium rolfsii*) and damping-off (*Pythium* sp.) are a significant bottleneck in the production of tomato and responsible for heavy economic losses.

In Algeria, *Alternaria* blight of tomato caused by *Alternaria alternata* is ruining this crop severely and causes 46-90 per cent blight disease intensity with huge fruit yield losses (Bessadat *et al.* 2014). *Alternaria* blight (*Alternaria alternata*) is one of the most dangerous diseases in Rajasthan and causes heavy fruit yield losses (Yadav *et al.* 2020). The fungal blight of tomato has become a major limiting factor in successfully cultivating tomato and causing yield loss that varies from 15 to 100% (Panthee and Chen, 2010; Kaur *et al.* 2016).

Management of *Alternaria* blight is complex because the pathogen has a wide host range, pathogenic variability and prolonged active phase of the disease cycle. The causal organism is soil-borne and spreads through the air, causing *Alternaria* blight, collar rot and fruit rot of tomato. Available methods for controlling *Alternaria* blight prevent long periods of wetness on the leaf surface, cultural practices, and host plant resistance with fungicides (Namanda *et al.* 2004; Kirk *et al.* 2005; Kumar and Srivastava, 2013). The application of fungicides is the most effective method of *Alternaria* blight control (Verma and Verma, 2010). However, the most important means of protecting plants against phytopathogenic fungi is synthetic fungicides (Saha *et al.* 2013; Abdel-Megeed *et al.* 2015). In India, Kumar *et al.* (2007) have tried many fungicides of triazoles and strobilurins groups and found them effective in managing *Alternaria* blight of tomato. In light of the above, the present investigation was carried out to know the efficacy of some novel fungicides belonging to triazoles and strobilurins groups for managing *Alternaria* blight of tomato.

MATERIALS AND METHODS

Experimental site

The experiment was carried out during *zaid* season (Feb. to May) for two consecutive years

(2017 and 2018) at S.K.N. College of Agriculture (SKNAU), Jobner, Jaipur (Rajasthan). Jobner is situated at latitude 26°5' N, the longitude of 75°20' E and an altitude of 427 meters above MSL (mean sea level). The region falls under the semi-arid eastern plain (Agro Climatic Zone- III A) of Rajasthan. All the recommended agronomic practices of the area were followed to raise the crop in a field.

Isolation, identification, preparation of Alternaria alternata inoculum and pathogenicity

Alternaria alternata was isolated from the infected leaves of tomato crop growing at Horticulture Farm, S.K.N. College of Agriculture Jobner, Jaipur, India. To prepare the *Alternaria alternata* inoculum, all the glassware was cleaned with potassium dichromate sulphuric acid solution, washed with sterilized water, and sterilized in a hot air oven at 160 °C for two hrs. Potato dextrose agar (PDA) medium was sterilized by autoclaving at 1.045 kg cm² pressure for 20 min. Leaves of diseased plants of tomato were first washed under the tap water and then cut into small pieces along with healthy portions. These pieces were surface sterilized using 1% sodium hypochlorite solution for 1 min. After three consecutive washings with sterilized distilled water, the pieces were transferred to autoclaved potato dextrose agar medium in Petri plates and incubated at 25±1 °C in biological oxygen demand (BOD) incubator for seven days in the dark. The fungal colonies emanating from bits were examined on the 7th day of incubation. Pure culture of the fungus was obtained through single spore technique. Monoconidial culture established in this way was maintained by periodical transfer on PDA slants. After purification, the fungus was allowed to sporulate. The sporulating pure culture was identified as *Alternaria* sp. on the basis of morphological features (Rangaswami and Rao, 1957; and Simmons, 1965). The culture of *Alternaria* sp. had long chains of conidia on the conidiophores and these were pale brown to olive brown in colour. The spores were large and appear dark brown with short beak. The identification of *Alternaria* sp. up to species level was confirmed from Indian Type Culture Collection (ITCC), Division of Plant Pathology, Indian

Agricultural Research Institute (IARI), New Delhi, with ID No. 9926.15. The pathogenicity of the pure culture was proved following Koch's postulate.

***In vitro* efficacy of fungicides on mycelial growth of the pathogen**

Seven systemic and non-systemic fungicides (hexaconazole, propiconazole, azoxystrobin, tebuconazole + trifloxystrobin, propineb, carbendazim + mancozeb and mancozeb) were tested to assess the anti-mycotic behaviour at three concentrations (100, 300 and 500 ppm) against *Alternaria alternata* by poisoned food technique (Schmitz, 1930). The requisite quantity of each fungicide was incorporated in a sterilized two per cent PDA medium, thoroughly mixed by shaking before pouring into sterilized Petri plates and were allowed to solidify. These Petri plates were inoculated with a 5 mm dia. disc of the seven-day-old culture of the pathogen in the centre of the plate and incubated at 25 ± 1 °C. Each treatment was replicated thrice with suitable control. Colony diameter was measured on the 7th day of incubation. Per cent mycelial growth inhibition was calculated as per the following formula (Bliss, 1934).

$$\text{Per cent growth inhibition} = \frac{C - T}{C} \times 100$$

Where,

C = Diameter of the colony in control (average of both diagonals)

T = Diameter of the colony in treatment (average of both diagonals)

Efficacy of fungicides in open field conditions

For managing *Alternaria* blight of tomato under field conditions, the aforesaid fungicides were also tested as foliar spray under artificial epiphytotic conditions during *zaid* season (Feb. to May) for two consecutive years (2017 and 2018) in randomized complete block design (RCBD) with three replications. During field experiments, one-month-old seedlings of a highly susceptible "Abhilash" variety of tomato (Seminis Vegetable Seeds India Pvt. Ltd., Maharashtra, India) were

transplanted in the second week of February during both years. The crop was raised in 2.4 m x 2.0 m plots with row-to-row and plant-to-plant distances of 60 cm x 45 cm. All the recommended agronomic practices were followed to raise the crop. The seven-day-old inoculum (5×10^5 conidia/ml), multiplied on PDA, was sprayed on the 30th day of transplanting. Congenial conditions for disease development were created by regularly applying light irrigations through sprinkler systems after inoculation of the pathogen.

Two foliar applications of all the tested fungicides were applied in the field at the rate of 0.2 per cent of the commercial product. The first spray was applied at the disease initiation at 45 and the second at 60 days after transplanting (DAT) in both years. One untreated control was maintained where only ordinary water was sprayed. As per the following details, the observations on disease intensity were recorded at 75th DAT by using the 0-5 disease rating scale of Horsfall and Barratt (1945) (Table 1), while per cent disease intensity (PDI) was calculated as per Wheeler (1969) and yield recorded up to the harvest of the crop (105 DAT).

$$\text{PDI} = \frac{\text{Sum of individual ratings}}{\frac{\text{The number of leaves observed}}{\text{Maximum disease grade}}} \times 100$$

The per cent disease control (PDC) over control was calculated as follows:

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

Statistical analysis

In the laboratory experiment, Petri plates were arranged in a completely randomized design (CRD), while under field experiments, it was arranged in a randomized complete block design (RCBD) with three replications. All the data collected during these investigations were entered in MS Excel (2007). The data were analyzed by two-way analysis of variance (ANOVA) after angular transformation. The treatment means were compared using the Fisher-LSD test at a 0.05 level of significance.

Table 1 : Standards for the assessment of disease severity

Disease rating/grade	Per cent leaf area affected	Description of the symptoms
0	-	Leaves free from infection
1	0.1-5.0	Small irregular spots covering 0.1- 5% of leaf area
2	5.1-10.0	Small irregular brown spots with concentric rings covering 5.1 - 10% leaf area
3	10.1-25.0	Lesions enlarging, irregular brown with concentric rings covering 10.1 - 25% leaf area
4	25.1-50.0	Lesions coalesce to form irregular and appear as a typical blightsymptom covering 25.1- 50% leaf area
5	> 50.0	Lesions coalesce to form irregular and appear as a typical blight symptom covering more than 50% leaf area

Table 2 : Efficacy of fungicides on mycelial growth of *Alternaria alternata* by poisoned food technique

Fungicides	Per cent inhibition of mycelial growth*			
	100 ppm	300 ppm	500 ppm	Mean
Propineb	55.77 (48.31)**	61.22 (51.48)	66.66 (54.73)	61.21 (51.48)
Hexaconazole	94.44 (76.36)	100 (90.00)	100 (90.00)	98.81 (83.74)
Propiconazole	79.77 (63.27)	84.33 (66.68)	100 (90.00)	88.03 (69.76)
Azoxystrobin	59.33 (50.38)	64.55 (53.46)	71.66 (57.84)	65.18 (53.84)
Mancozeb	65.66 (54.13)	74.66 (59.78)	85.55 (67.66)	75.62 (60.41)
Tebuconazole +Trifloxystrobin	88.86 (70.50)	94.4 (76.36)	100 (90.00)	94.36 (76.26)
Carbendazim + Mancozeb	71.88 (57.98)	80.11 (63.51)	86.77 (68.67)	79.58 (63.14)
Control	0.00	0.00	0.00	0.00
		SEm±	CD (p=0.05)	
	F	1.39	3.86	
	C	0.85	2.36	
	FxC	2.41	6.68	

*Average of three replications, **Values in parenthesis are angular transformed as:DEGREES(ASIN(SQRT(% value/100))); F=Fungicides, C=Concentrations, SEm±=Standard error of the mean, CD= Critical difference, F x C= Fungicides x Concentrations

RESULTS AND DISCUSSION

In vitro efficacy of fungicides

Analysis of *in vitro* data (Table 2) showed that an increased concentration of the respective fungicide caused increased inhibition of the mycelial growth of the pathogen. Among tested fungicides, hexaconazole caused the highest

inhibition of mycelial growth (94.44%, 100% and 100%) of *Alternaria alternata* at all the tested concentrations i.e. at 100 ppm, 300 ppm and 500 ppm concentrations, respectively, followed by tebuconazole +trifloxystrobin with inhibition of 88.86, 94.40 and 100 % at 100,300 and 500 ppm, respectively. Propineb was found least effective at all concentrations against *Alternaria alternata*. At 100 ppm, propineb and azoxystrobin were

Table 3: Efficacy of fungicides on *Alternaria* blight of tomato and yield under artificial epiphytotic conditions

Fungicides	Conc. (%)	Per cent disease intensity*		Pooled	Reduction in PDI over control (%)	Yield (q/ha)*		Pooled	Increase in yield over control (%)
		2017	2018			2017	2018		
Propineb	0.2	40.13 (39.31)**	28.61 (32.34)	34.37 (35.89)	48.03	161.51	179.65	170.58	49.90
Hexaconazole	0.2	15.67 (23.32)	11.13 (19.49)	13.40 (21.47)	79.74	211.20	217.83	214.52	88.51
Propiconazole	0.2	21.13 (27.37)	15.71 (23.35)	18.42 (25.42)	72.14	195.13	210.80	202.97	78.37
Azoxystrobin	0.2	33.13 (35.14)	25.67 (30.44)	29.40 (32.83)	55.55	173.67	185.56	179.62	57.85
Mancozeb	0.2	28.42 (32.22)	23.98 (29.32)	26.70 (31.11)	59.63	178.18	194.90	186.54	63.93
Tebuconazole +Trifloxystrobin	0.2	17.31 (24.59)	13.98 (21.96)	15.65 (23.30)	76.33	198.80	216.20	207.50	82.35
Carbendazim + Mancozeb	0.2	24.73 (29.82)	18.17 (25.23)	21.45 (27.59)	67.57	190.22	197.10	193.66	70.19
Control	-	69.11 (56.24)	63.17 (52.64)	66.14 (54.42)	-	111.40	116.17	113.79	-
SEm _±		1.17	0.98	1.08		4.59	4.83	4.71	
CD (p=0.05)		3.55	2.97	3.26		14.13	14.87	14.50	

*Average of three replications, **Values in parenthesis are angular transformed as: DEGREES (ASIN (SQRT(% value/100))); PDI = Per cent disease intensity, SEm_± = Standard error of the mean, CD = Critical difference,

found at par with each other with 55.77% and 59.33% of mycelial growth inhibition. At 500 ppm, mancozeb and carbendazim + mancozeb were found at par with each other with 85.55% and 86.77% of mycelial growth inhibition. Similar observations were recorded by Sharma and Gaur (2009) and Kumar and Singh (2017) against *Alternaria solani* causing early blight disease of tomato and recorded that fungicide hexaconazole 5% EC was the most effective, followed by thiafluzamide 24% SC and trifloxystrobin 25% w/w + tebuconazole 50% WG at 500ppm. Our results are also similar to the findings of Nikam *et al.* (2014). They concluded that propiconazole and penconazole were found effective in inhibiting mycelial growth (94.4%) followed by hexaconazole (94.19%), difenoconazole (86.84%) and benomyl (82.34%). In line of present findings, Jakatimath *et al.* (2017) have also recorded similar results while working with *Alternaria alternata*, the incitant of fruit rot of brinjal.

Efficacy of fungicides in open field conditions

In the present investigation, as far as disease control and yield are concerned, all the fungicides tested performed better over check in reducing

disease intensity and increasing yield under artificial inoculated field conditions. Two years of pooled results (Table 3) revealed that all the fungicides were significantly effective in reducing the *Alternaria* blight disease over control. The lowest disease intensity (13.40 %) with higher disease reduction (79.74%) was recorded with twofoliar applications of hexaconazole (@0.2%) followed by tebuconazole +trifloxystrobin (15.65% and 76.33%, respectively) Propineb was found to be the least effective (34.37%). Statistically hexaconazole and tebuconazole +trifloxystrobin were at par to each other in minimizing disease and in increasing fruit yield. Maximum fruit yield (214.51 q/ha) was harvested with hexaconazole followed by tebuconazole +trifloxystrobin (207.50 q/ha) in comparison to check (113.79 q/ha). Our findings are similar to the results of earlier researchers on the early blight of tomato (Kumar *et al.* 2007; Jambhulkar *et al.* 2012; Kumar and Barnwal, 2016); on *Alternaria* leaf spot of cotton (Meena and Ratnoo, 2013), on fruit rot of chilli (Ginoya and Gohel, 2015) and on *Alternaria* blight of tomato (Yadav *et al.* 2020). Jambhulkar *et al.* (2012) sprayed azoxystrobin 23% SC on tomato plants and recorded promising results in reducing disease severity by 38.9 per cent. Meena and Ratnoo (2013) also obtained minimum disease

severity by foliar application of hexaconazole and mancozeb followed by copper oxychloride in the management of *Alternaria* leaf spot of cotton. Kumar and Barnwal (2016) recorded the lowest disease severity (12.0%) by providing three sprays of hexaconazole 4% + zineb 68% (@0.1%) along with higher fruit yield (219.8 q/ha). Concerning tomato blight control and consumer health issues, Liang *et al.* (2012) conducted experimentation by applying hexaconazole (@ 150g a.i. per square hectometer) for three or four times with an interval of 7 days in the field on tomatoes. They observed its effectiveness in controlling blight disease and residues below 0.1 mg/kg MRL (maximum residue limit) on the seventh day of the last application which means that the application of hexaconazole on tomato plants is safe for consumers.

Two years' study revealed that foliar application of hexaconazole provided higher fruit yield by reducing disease severity. It may, thus, be concluded that two sprays of hexaconazole (@ 0.2%) or tebuconazole +trifloxystrobin at 15 days intervals reduced disease intensity effectively with increased fruit yield and profitability.

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DECLARATION

Conflict of interest. The authors declare no conflict of interest.

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