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In-vitro Efficacy of Fungicides against *Alternaria alternata* causing Blight Disease of Tomato (*Solanum lycopersicum* L.)

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A B S T R A C T

Tomato is world's one of the largest growing vegetable crop and ranks first among processing crops. It's production suffers with many biotic and abiotic factors. Leaf blight disease caused by different species of Alternaria is one of the most dreadful diseases of tomato, causing huge economic losses. The present study was conducted in order to evaluate the efficacy of different fungicides against *Alternaria alternata*, one of cause for blight disease of tomato. The efficacy of fungicides *viz.*, Propineb 70% WP, Hexaconazole 5% EC, Propiconazole 25% EC, Azoxystrobin 23% SC, Mancozeb 75% WP, Tebuconazole (50%) + Trifloxystrobin (25%) WG and Carbendazim (12%) + Mancozeb (63%) WP was tested at different concentrations *viz.*, 100, 150, 200 and 500 ppm against *A. alternata* on PDA by poisoned food technique. It was concluded that maximum mycelial growth inhibition (97.96%) was recorded by Hexaconazole, followed by Tebuconazole + Trifloxystrobin (94.17%). However, Propineb recorded least per cent inhibition of mycelium growth at all concentrations with a mean percent growth inhibition of 60.83 percent.

Keywords

Tomato, *Alternaria alternata*, Fungicides, Percent mycelial growth inhibition

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Introduction

Tomato (*Solanum lycopersicum* L., syn. = *Lycopersicon esculentum* Mill.) belongs to Solanaceae family and is one of the most remunerable and widely grown vegetables in the world. Among the vegetables, tomato ranks next to potato in world acreage and first among the processing crops. Tomato is a native from Peruvian and south American region but consumed world widely because it contains good amount of antioxidants and vitamins *i.e.* A, C and E,, which are necessary for metabolic activities and thus for maintaining good human health. Adaptability in relation to different habitats and its high nutritive value made tomato more popular in recent years (Olaniyi *et al.*, 2010). India is the second largest producer of tomato crop after China, having an area of 778 thousand hectares with a production of 19.4 million metric tonnes (Anonymous, 2020). In India major tomato growing states are, Andhra Pradesh, Madhya Pradesh, Karnataka, Odisha, West Bengal, Chhattisgarh, Telangana, Bihar, Gujarat, Uttar Pradesh and Rajasthan. In Rajasthan, tomato crop is mainly grown in Jaipur, Dausa, Alwar, Tonk, Dholpur, Bharatpur, Chittorgarh and Kota districts, with an area of 18.12 thousand hectares and annual production of 88.73 thousand metric tonnes during 2017-18 (Anonymous, 2019). Various factors are responsible for low yield and among them diseases are of most concern. Tomato crop is prone to different fungal, bacterial, nematode and viral diseases. Introduction of some high yielding cultivars of tomato have replaced most of the conventional cultivars, having natural resistance against many pathogens. Monoculture and favourable climatic conditions attracts several fungal diseases. Nearly 200 diseases have been reported world widely to infect tomato. Important fungal diseases like Alternaria blight (*Alternaria alternata*), early blight (*Alternaria solani*), Late blight (*Phytophthora infestans*), Septoria leaf blight (*Septoria lycopersici*), Powdery mildew (*Oidiopsis taurica*), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), Collar rot (*Sclerotium rolfsii*) and Damping off (*Pythium sp*.) cause considerable losses in tomato crop. Among all fungal diseases, leaf blight disease of tomato caused by *Alternaria alternata* is one of the most wide spread and devastating disease occurring worldwide in major tomato growing area (Atherton and Rudich, 1986; Akhtar *et al.*, 1994; Dun-chun *et al.*, 2016; Adhikari *et al.*, 2017). Thus, the present study was conducted at College of Agriculture, Ummedganj-Kota (Agriculture University- Kota) during 2019-2020, in order to evaluate the efficacy of different fungicides at four different concentrations (100, 150, 200 and 500 ppm) against *Alternaria alternata* causing blight disease of tomato by using poison food technique (Nene and Thapliyal, 2018).

Materials and Methods

Collection of diseased samples

Infected diseased samples showing typical symptoms of Alternaria blight were collected during month of January-February, 2019 from the tomato fields of Jalkhera village nearby Agricultural Research Station, Ummedganj (Kota). Samples were brought into Department of Plant Pathology, College of Agriculture, Ummedganj, (Kota) for isolation and further studies.

Isolation and identification of fungus

For isolation of the pathogen standard tissue isolation technique was followed. The pathogen was isolated from infected tomato leaves. These leaves were cut into small pieces along with growing margins of disease about 1.5-2 mm. Then these pieces were surface sterilized with 0.1% $HgCl_2$ solution for one minute. Such pieces were washed thoroughly by sterile distilled water three times to remove the traces of mercuric chloride solution, and then aseptically transferred to sterilized potato dextrose agar (PDA) plates. These plates were incubated at 25±1 °C for three days. Later, the bits of fungal growth were transferred to another PDA plates. These petri plates were incubated at 25±1°C for one week to check the sporulation for further studies. Pure culture was obtained with the help of single spore technique by incubating at about 28°C for seven days under regular observation to get rid of contamination. The isolated fungus was identified on the basis of morphological characters. The culture was also sent to ITCC, Division of Plant Pathology, IARI, New Delhi for further confirmation or identification of fungus with (ID No. 11, 291.20).

In-vitro evaluation of fungicides against *Alternaria alternata* by poison food technique

Fungicides listed in Table 1 were tested against *Alternaria alternata* at different concentrations of 100, 150, 200 and 500 PPM on Potato dextrose agar (PDA) medium using poisoned food technique (Nene and Thapliyal, 2018). Potato dextrose agar medium (100ml) was sterilized in conical flask of 250 ml capacity. The required amount of the fungicides separately incorporated aseptically in molten PDA to get a requisite concentration of that chemical. To avoid bacterial contamination little amount Streptomycin was added to each flask before pouring.

The amended medium was then poured (25 ml in each plate) in prior sterilized Petri plates (90mm). Medium without any chemical served as control. A 6 mm mycelial disc of test fungus was cut with the help of sterilized cork borer from margin of 5 days old culture and then placed centrally in Petri plates. The disc was placed in inverted position to allow contact of fungus with medium. Three replications were maintained for each treatment.

The inoculated Petri plates were incubated in the BOD incubator at 25±1ºC and the colony diameter of the pathogen was measured after 7 days of incubation with the help of scale in millimetre. Per cent growth inhibition, under the influence of different fungicides at different concentrations was calculated over control by using the formula given by Vincent (1947). The data were analyzed statistically.

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

Where, I = per cent growth inhibition, C = growth in control, T = growth in treatment.

Results and Discussion

The efficacy of fungicides was assessed *in vitro* at four concentrations *viz.*, 100, 150, 200 and 500 ppm against *Alternaria alternata* on PDA medium by poison food technique (Table 2 and Figure 1 and 2). Data suggested that increase in concentration of fungicides resulted in inhibition of mycelium growth of fungus. Among these fungicides, Hexaconazole was found superior in inhibiting the growth of *Alternaria alternata* at all four concentrations with a mean percent inhibition of 97.96 percent followed by Tebuconazole + Trifloxystrobin (94.17%). However, propineb recorded least percent inhibition of mycelium growth at all concentration with a mean 63.33 percent growth inhibition. At 100 ppm, Hexaconazole showed maximum percent inhibition (91.85) followed by Tebuconazole +Trifloxystrobin (84.44%) and least percent inhibition was recorded by propineb (57.41%). At 150 ppm, Hexaconazole showed cent percent inhibition followed by Propiconazole (93.33 %) and least percent inhibition was recorded by propineb (61.11%). At 200 and 500 ppm, Hexaconazole, Propiconazole, Azoxystrobin and Tebuconazole + Trifloxystrobin showed cent percent inhibition of mycelium growth followed by Carbendazim + Mancozeb with 78.52 percent and 84.44 percent inhibition respectively. Least percent inhibition was recorded for Propineb (65.19 and 69.63%). These results are in conformity with the findings of those reported earlier by several workers. Systemic fungicides *viz.*, Propiconazole, Penconazole, Difenconazole and Hexaconazole were reported to cause significant inhibition of various species of Alternaria including *A. solani, A. helianthi and A. alternata* (Amaresh and Nargund, 2004; Pawar, 2004; Kharkwal *et al.,* 2006; Akbari and Parakhia, 2007; Firake, 2008; Bhaskar and Lukose, 2012; Rajani and Rakholia, 2012; Jakatimath *et al.,* (2017).

Sharma (2019) found that fungicide Hexaconazole (98.81%) is the most effective in inhibiting the mycelium growth of *A. alternata* followed by Tebuconazole + Trifloxystrobin (94.36%). However, Yadav (2016) reported that Tebuconazole + Trifloxystrobin (87.00%) found most effective followed by Hexaconazole.

Table.1 Details of fungicides and concentration used for investigation

S. No.	Technical ingredient	Trade name	Concentration (ppm)
1.	Propineb 70% WP	ANTRACOL	100, 150, 200 & 500
2.	Hexaconazole 5% EC	CONTAF	100, 150, 200 & 500
3.	Propiconazole 25% EC	TILT	100, 150, 200 & 500
4.	Azoxystrobin 23% SC	AMISTAR	100, 150, 200 & 500
5.	Mancozeb 75% WP	SPARSH	100, 150, 200 & 500
6.	Tebuconazole (50%) + Trifloxystrobin (25%) WG	NATIVO	100, 150, 200 & 500
7.	Carbendazim (12%) + Mancozeb (63%) WP	SAAF	100, 150, 200 & 500
	Control		

Table.2 *In-vitro* efficacy of fungicides against *Alternaria alternata* by poisoned food technique

Treatment / Fungicides	**Percent mycelial growth inhibition of *Alternaria alternata***				
	100 ppm	**150 ppm**	**200 ppm**	**500 ppm**	**Mean**
T_1: Propineb 70% WP	57.41 * (49.26) **	61.11 (51.42)	65.19 (53.84)	69.63 (56.56)	63.33 (52.77)
T_2: Hexaconazole 5% EC	91.85 (73.47)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	97.96 (85.87)
T_3: Propiconazole 25% EC	77.78 (61.88)	93.33 (75.17)	100.00 (90.00)	100.00 (90.00)	92.78 (79.26)
T_4: Azoxystrobin 23% SC	79.63 (63.18)	84.07 (66.50)	100.00 (90.00)	100.00 (90.00)	90.93 (77.42)
T_5: Mancozeb 75% WP	58.15 (49.69)	65.56 (54.07)	72.59 (58.45)	80.74 (64.01)	69.26 (56.55)
T_6: Tebuconazole (50%) + Trifloxystrobin (25%) WG	84.44 (66.78)	92.22 (73.91)	100.00 (90.00)	100.00 (90.00)	94.17 (80.17)
T_7: Carbendazim (12%) + Mancozeb (63%) WP	67.04 (54.97)	75.19 (60.13)	78.52 (62.40)	84.44 (66.78)	76.30 (61.07)
T_8: Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Concentrations Mean	64.54 (52.40)	71.44 (58.90)	77.04 (66.84)	79.35 (68.42)	73.09 (61.64)
	Treatment	**Concentration**		**T X C**	
S Em. ± =	**0.25**	**0.17**		**0.50**	
C.D. at 0.05% =	**0.71**	**0.50**		**1.43**	

*Mean of three replications;
**Figures in parentheses are Arc sine transformed values

Fig.1 *In-vitro* efficacy of Fungicides against *Alternaria alternata* at different concentration

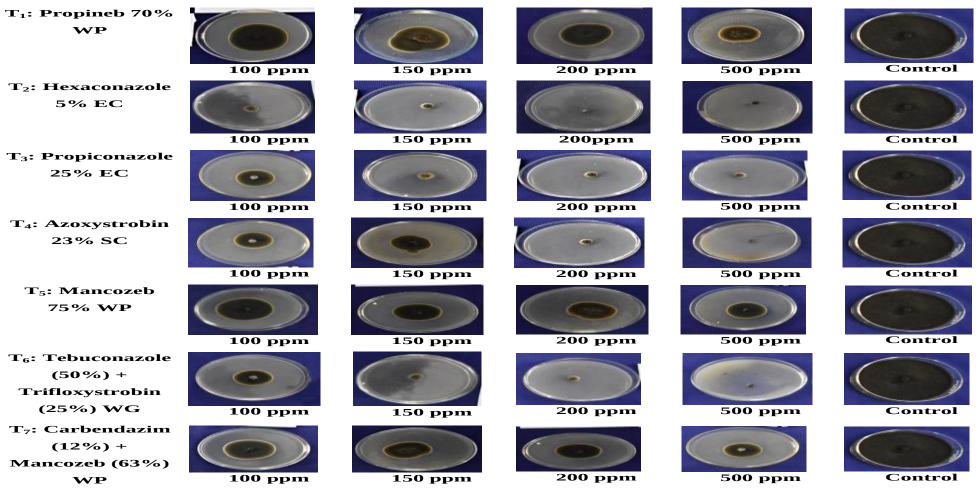
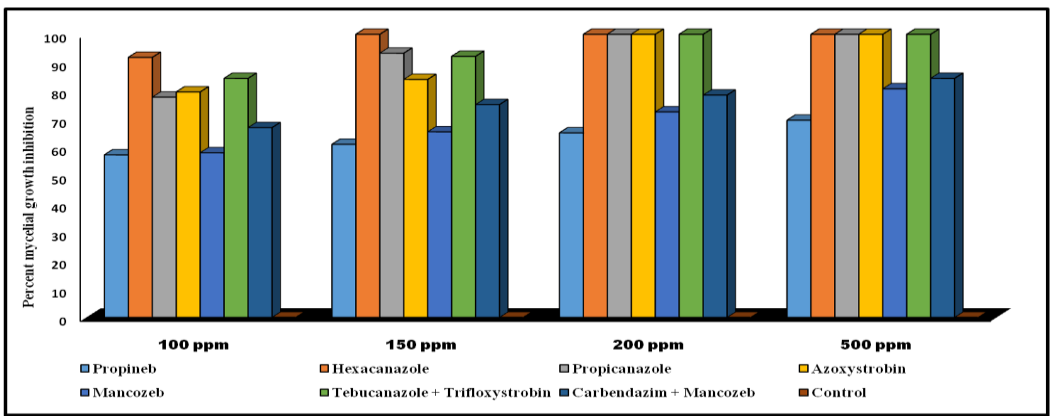


Fig.2 *In-vitro* efficacy of Fungicides against *Alternaria alternata* at different concentration



In conclusion the current experiment was conducted to unveil the efficacy of fungicides against radial growth of pathogen *in vitro* and it can be concluded that among seven fungicides used for this study, Hexaconazole found to be superior in inhibiting fungal growth under laboratory condition followed by Tebuconazole + Trifloxystrobin. Fungicide application is one of the sharp tool for management of fungal diseases like leaf blight of tomato caused by *Alternaria alternata*. Conclusively, judicious and need based use of fungicides along with other management practices may give an eco friendly solution to fungal diseases like leaf blight of tomato caused by *A. alternata*.

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