



## ***In vitro* Screening of New Fungicide Molecules against *Alternaria* sp. Isolated from Tomato**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Early blight disease caused by *Alternaria* spp. is one of the major limiting factors in tomato-growing areas of the country, causing considerable yield loss. Early blight can be effectively managed by cultural practices, chemical fungicides, and through the use of resistant genotypes. Cultural practice alone are not sufficient for the control of early blight, hence several fungicides have been developed and tried for the management of early blight. In the present study, in order to develop effective management practices against the early blight of tomato, a total of 22 fungicides comprising six systemic, six contact and ten combi products were tested for their fungitoxicity against *Alternaria* sp. under *in vitro* by following the poisoned food technique. All the fungicides significantly reduced the radial mycelial growth of *Alternaria* spp. Based on the maximum inhibition at the lowest concentration, the six fungicides *i.e.* Hexaconazole 5% EC (50.00), Propiconazole 25 % EC (65.00), Tebuconazole 25.9% EC(53.03),Mancozeb 75 % WP (54.44), Propineb 70 % WP (50.00), Copper oxychloride 50 % WP(63.15), Tricyclazole 45% W G + Hexaconazole 10 % WG(93.17), Fluxapyroxad 250 g/L + Pyraclostrobin 250 g/L SC (92.08)and Zineb 68% + Hexaconazole 4% WP(86.01)were recorded the best against *Alternaria* sp. compared to control.

**Keywords:** *Alternaria*; propiconazole; hexaconazole; fungicides.

## 1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the most important fruit and vegetable crop consumed in the world with estimated production of 21.0 mmt ha<sup>-1</sup> every year globally on an area of 841 thousand hectares [1]. The tomato crop is highly susceptible to infection caused by various pathogens. Among them the early blight disease caused by *Alternaria solani* (Ellis & Martin) is one of the main limiting factors in tomato-growing areas of the country, causing considerable yield loss [2]. The yield losses reported owing to this disease was estimated around 79% [3]. Several fungicides have been developed and tried for the management of early blight. Different groups of fungicides are available for the management of early blight of tomato. The efficacy of combi product, Azoxystrobin 11% + Tebuconazole 18.3% SC against early blight disease of tomato was demonstrated by [4]. Systemic fungicides like Hexaconazole 5% EC and Propiconazole 25 EC were also found to be significantly superior in suppressing the disease severity in tomato [5]. Among contact fungicides, Chlorothalonil 75 % WP and Copper oxychloride 50 % WP recorded the highest inhibition against *A. solani*.

The main purpose of this study was to evaluate different fungicide molecules against early blight of tomato caused by *Alternaria* spp.

## 2. MATERIALS AND METHODS

### 2.1 *In vitro* Evaluation of Fungicides against *Alternaria* spp.

The PDA medium was prepared and amended with the appropriate quantity of fungicides as per the concentration. Twenty milliliters of media amended with fungicides was added to sterile Petri plates and was inoculated with 9 mm mycelial disc of the pathogen. The control plates were maintained without fungicides. The inoculated plates were maintained at 27°C in a BOD incubator. The growth of the pathogen in fungicide amended plates was measured when the pathogen growth in control plates attained full growth. The radial growth and per cent inhibition in each concentration was calculated as per [6]. Inhibition per cent =  $[(C-T)/C] \times 100$ , Where, I= Per cent inhibition (%), C=Growth of test fungus in control (mm) and T=Growth of the test fungus in treatment (mm) was calculated.

## 2.2 Statistical Analysis

The statistical analysis that was used in the present investigation is CRD (Completely Random Design).

## 3. RESULTS AND DISCUSSION

*In vitro* evaluation of fungicides provides effective and preliminary information about fungicide efficacy against pathogens in a short period, and thus serves as a model for field testing. In the present investigation, the poison food technique was used for testing fungicide efficacy. Six systemic, six contacts and ten combi fungicides were screened for their efficacy against *Alternaria* spp. on PDA media. The screening of the fungicides revealed that all the test fungicides significantly inhibited the radial mycelial growth of *Alternaria* spp. at various tested concentrations. Among the systemic fungicides tested at different concentrations, Propiconazole 25% EC recorded the highest per cent inhibition of 65.00%, followed by Tebuconazole 25.9% EC and Hexaconazole 5 % EC with per cent inhibition of 53.03%, 50.00%, respectively at 100 ppm concentration (Table 1 and Plate 1). The fungicidal activity of triazoles was attributed to the inhibition of sterol biosynthesis. The results were in agreement with findings of [7] who documented the efficacy of propiconazole, Tricyclazole and Hexaconazole found to be effective against *Alternaria solani* *in vitro*. Further, Arshad et al. [8] reported the cent per cent efficacy of Propiconazole 25% EC against *Alternaria* *in vitro* at 100 ppm.

Among the contact fungicides tested against *Alternaria* *in vitro*, Copper oxychloride 50% WP was yielded the maximum reduction (63.15%) in the mycelial growth at 500 ppm, followed by Mancozeb 75% WP and Propineb 70 % WP with per cent inhibition of 54.44% and 50.00%, respectively (Table 2 and Plate 2). The efficacy of copper against *Alternaria* sp. is attributed to its multi-site activity. The results conformed with the findings of [9,10] who reported the efficacy of Copper oxychloride 50% WP against the early blight of tomato.

In the present study, combi-products viz., Tricyclazole 45% W G + Hexaconazole 10% WG (93.17%) , Fluxapyroxad 250 g/L + Pyraclostrobin 250 g/L SC (92.08%) and Zineb 68% + Hexaconazole 4% WP (86.0%) recorded

the maximum inhibition of the growth of fungus at 500 ppm (Table 3 and Plate 3). The fungicidal activity of combi products with two different modes of action leads to the synergistic effect with high efficacy for the control of the pathogen. The results of the present investigation were in

conformation with the findings of [11,7] who documented combi fungicide i.e. Tricyclazole 45% W G + Hexaconazole 10 % WG, Fluxapyroxad 250 g/L + Pyraclostrobin 250 g/L SC and Zineb 68% + Hexaconazole 4% WP efficacy against *Alternaria solani* *in vitro*.

**Table 1. Systemic fungicides on the growth of *Alternaria* sp. (A7)**

Fungicides	*Inhibition (%) at different concentration (ppm)		
	100	250	500
Azoxystrobin 23% EC	23.61*±3.25** (29.0)	25.19±4.07 (30.1)	51.11±3.04 (45.6) ***
Carbendazim 50% WP	25.74±3.33 (30.5)	27.41±0.29 (31.6)	68.08±1.72 (55.6)
Tricyclazole 75% WP	48.00±0.75 (46.7)	62.04±1.53 (47.3)	95.00±1.50 (77.2)
Tebuconazole 25.9% EC	53.03±2.18 (45.0)	54.04±1.69 (47.9)	54.50±1.79 (47.6)
Hexaconazole 5% EC	50.00±3.50 (43.9)	55.00±3.04 (52.0)	73.89±1.32 (59.3)
Propiconazole 25% EC	65.00±4.58 (53.8)	68.22±2.12 (55.7)	72.22±2.00 (58.2)
<b>SE.m ±</b>	<b>1.25</b>	<b>0.98</b>	<b>0.89</b>
<b>CD @ 1%</b>	<b>3.85</b>	<b>3.03</b>	<b>2.75</b>

\*Mean of three replication

\*\*In a column numerical value followed by ± represents standard deviation

\*\*\*Figures in the parenthesis indicates the arcsine transformed values



**Plate 1. Systemic fungicides on the growth of *Alternaria* sp.**

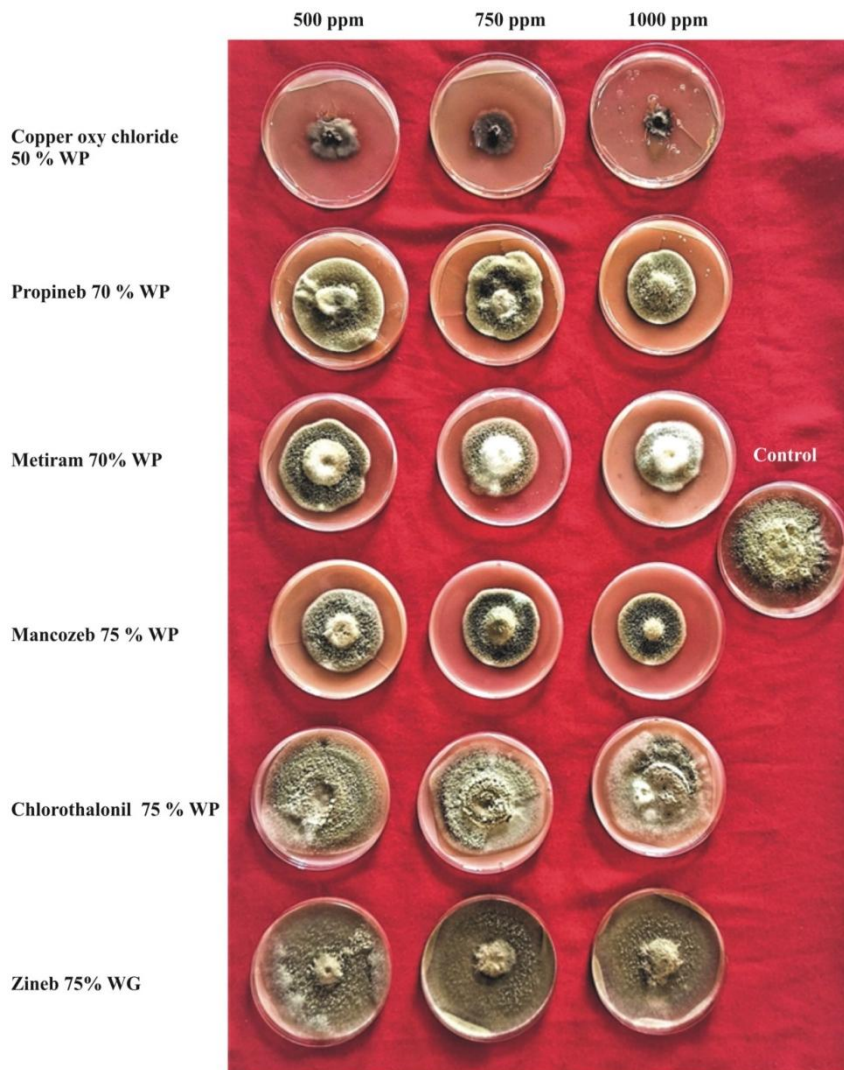
**Table 2. Contact fungicides on the growth of *Alternaria* sp. (A7)**

Fungicides	Inhibition (%) at different concentration (ppm)		
	500	750	1000
Zineb 75% WG	8.52*±3.21** (16.7)	12.41±1.15 (20.6)	18.89±36.53 (25.7) ***
Chlorothalonil 75% WP	20.00±1.80 (26.5)	23.70±1.04 (29.1)	25.56±33.51 (30.4)
Mancozeb 75% WP	54.44±0.87 (47.6)	56.30±0.29 (48.6)	60.00±18.03 (50.8)
Propineb 70% WP	50.00±2.29 (45.0)	55.00±2.18 (47.9)	58.15±18.86 (49.7)
Metiram 70%WP	45.56±1.32 (42.4)	47.00±2.01 (43.3)	47.47±23.71 (43.5)
Copper oxychloride 50% WP	63.15±2.47 (52.6)	67.04±4.25 (55.0)	73.15±12.47 (58.8)
<b>SE.m ±</b>	<b>1.14</b>	<b>0.87</b>	<b>0.85</b>
<b>CD @ 1%</b>	<b>3.51</b>	<b>2.69</b>	<b>2.61</b>

\*Mean of three replication

\*\*In a column numerical values followed by ± represents standard deviation

\*\*\*Figures in the parenthesis indicates the arcsine transformed values



**Plate 2. Contact fungicides on the growth of *Alternaria* sp.**

**Table 3. Combi fungicides on the growth of *Alternaria* sp. (A7)**

Fungicides	Inhibition (%) at different concentration (ppm)					
	500		750		1000	
Azoxystrobin 11% + Tebuconazole 18.3% w/w SC	79.01 $\pm$ 5.22**	(62.9)	79.38 $\pm$ 5.66	(63.2)	80.00 $\pm$ 3.94	63.5***
Tricyclazole 45% W G + Hexaconazole 10 % WG	93.17 $\pm$ 0.42	(74.9)	95.00 $\pm$ 0.50	(77.1)	95.56 $\pm$ 0.56	(77.8)
Tricyclazole 18 % + Mancozeb 62 % WP	40.00 $\pm$ 0.87	(39.2)	45.19 $\pm$ 5.58	(42.2)	58.70 $\pm$ 1.16	(50.0)
Metiram 55% + Pyraclostrobin 5% WG	75.37 $\pm$ 1.89	(60.3)	77.59 $\pm$ 1.04	(61.8)	79.44 $\pm$ 0.56	(63.0)
Zineb 68% + Hexaconazole 4% WP	86.01 $\pm$ 1.66	(68.1)	87.00 $\pm$ 5.07	(69.1)	93.01 $\pm$ 1.09	(74.7)
Carbendazim 12% + Mancozeb 63% WP	28.05 $\pm$ 2.60	(32.0)	43.70 $\pm$ 3.33	(41.4)	56.40 $\pm$ 3.92	(48.7)
Fluxapyroxad 250 g/L + Pyraclostrobin 250 g/L SC	92.08 $\pm$ 1.52	(73.7)	93.33 $\pm$ 0.50	(75.0)	93.89 $\pm$ 0.96	(75.7)
Tebuconazole 25% + Trifloxystrobin 50% WG	80.01 $\pm$ 5.32	(63.6)	82.00 $\pm$ 0.72	(64.9)	86.11 $\pm$ 2.13	(68.2)
Azoxystrobin 20% + Difenconazole 12.5% w/w SC	84.01 $\pm$ 1.64	(66.5)	84.07 $\pm$ 2.02	(66.5)	89.04 $\pm$ 5.43	(71.0)
Famoxadone 16.6% + Cymoxanil 22.1% SC	67.02 $\pm$ 1.54	(55.0)	68.01 $\pm$ 1.09	(55.6)	69.04 $\pm$ 0.96	(56.2)
<b>SE.m <math>\pm</math></b>	<b>1.27</b>		<b>1.46</b>		<b>1.21</b>	
<b>CD @ 1%</b>	<b>3.75</b>		<b>4.31</b>		<b>3.57</b>	

\*Mean of three replication

\*\*In a column numerical values followed by  $\pm$  represents standard deviation

\*\*\*figures in the parenthesis indicates the arcsine transformed values



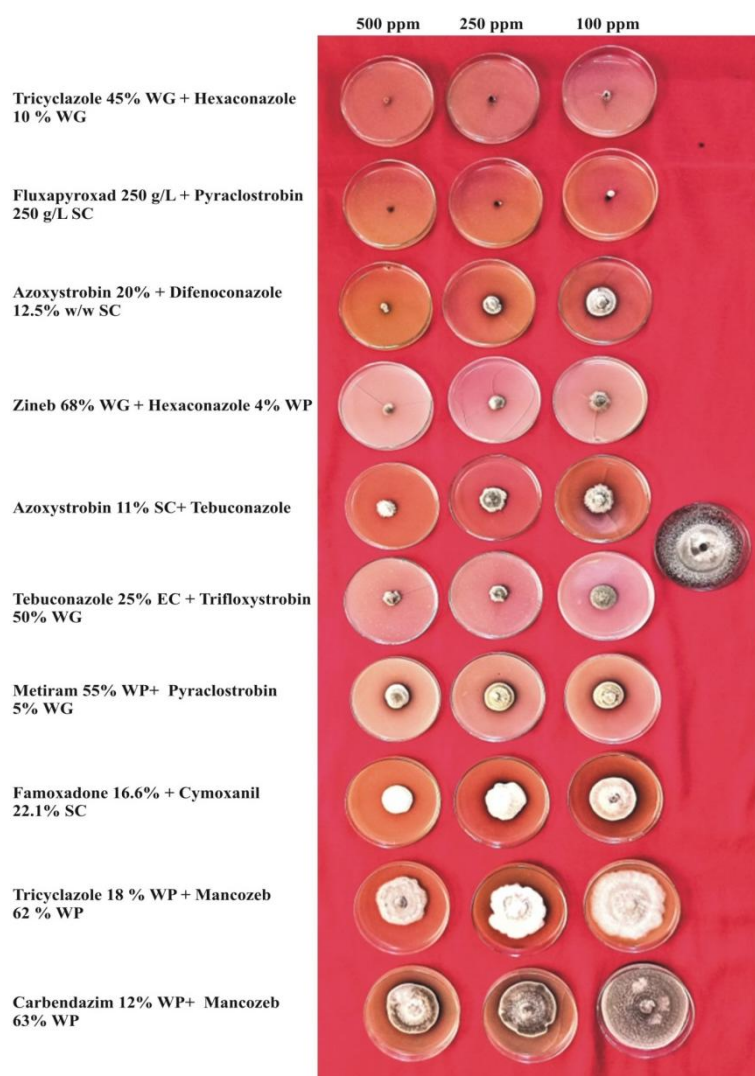


Plate 3. Combi fungicides on the growth of *Alternaria* sp.

#### 4. CONCLUSION

Studies on the comparative efficacy of fungicides against *Alternaria* under *in vitro* conditions were carried out. Among the fungicides, Propiconazole 25% EC, Copper oxychloride 50% WP and Tricyclazole 45% W G + Hexaconazole 10 % WG were found to be effective in arresting the growth of *Alternaria* sp *in vitro*.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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