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Evaluation of different fungicides against *Alternaria macrospora* (Zimm.) causing *Alternaria* leaf spot in cotton under *in vitro* condition

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Abstract
A laboratory experiment was conducted to study the efficacy of different fungicides against *A. macrospora.* The results revealed that among non-systemic fungicides, mancozeb 75% WP and zineb 75% WP completely inhibited the mycelial growth of pathogen at 2000 ppm concentration *in vitro.* Among systemic fungicides, propiconazole 25% EC at 100, 250 and 500 ppm concentrations completely inhibited the mycelial growth *in vitro*. Difenoconazole 25% EC at 500 and 250 ppm concentration remained equally effective with 87.23 and 84.27 percent mycelial growth inhibition, respectively. Whereas, among the ready-mixed fungicides fluxapyroxad 250 g/L + pyraclostrobin 250 g/L SC and tebuconazole 50% + trifloxystrobin 25% WG at 250, 500 and 1000 ppm concentrations completely inhibited the mycelial growth of test pathogen *in vitro* followed by azoxystrobin 18.2% + difenoconazole 11.4% SC and hexaconazole 4% + zineb 68% WP each at 1000 ppm concentration, respectively.

Keywords: Cotton, alternaria, fungicides, mycelial growth inhibition

Introduction
Cotton belongs to the family *Malvaceae* is the oldest among the commercial crops of the world providing fiber for clothing for the mankind. Among the various diseases, Alternaria leaf spot caused by *Alternaria macrospora* Zimm. is a major foliar disease of cotton which results in heavy defoliation and reduced seed cotton yield. The disease in field condition can be managed by using various fungicides. Recently, the available formulation of fungicides need to be test for their efficacy under laboratory condition before its application in the field. In this view, the present work carried out to study the effectiveness of various fungicides in inhibition of mycelial growth of the fungus using potato dextrose agar media.

Materials and Methods
Efficacy of different fungicides against *Alternaria macrospora in vitro*
An experiment was conducted at the laboratory of Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh to test the inhibitory action of different fungicides against *Alternaria macrospora*, a pathogen of *Alternaria* leaf spot of cotton. A mycelial growth inhibition activities of different fungicides were tested against the test pathogen *in vitro* by employing Poisoned Food Technique of Bagchi and Das (1968) using potato dextrose agar (PDA) as a germinating medium. Measured quantity of each fungicides required were incorporated into autoclaved PDA medium containing conical flask before solidification. They were thoroughly mixed by squiring for few seconds and then medium was poured into sterilized Petri plates (90 mm dia.) in equal quantity (20 ml per Petri plate) to form a uniform layer. An actively growing mycelial bit of 4 mm diameter from the periphery of five days old culture of *A. macrospora* was cut with the help of cork-borer were transferred under aseptic conditions in an inverted position over the solidified PDA medium containing Petri plates in the centre. Then Petri plates were incubated at 28$\pm$2 °C till control plate attains full growth and observations were recorded on linear mycelial growth in treated and control plates. Inoculated Petri plates containing PDA medium without fungicides served as control.
Each set of experiment comprised seven different treatments and three levels of concentrations (ppm). All the fungicides were evaluated against *A. macrospora* under laboratory condition by following Poisoned Food Technique. Among them, non-systemic fungicides at 500, 1000 and 2000 ppm; systemic fungicides at 100, 250 and 500 ppm and ready-mixed fungicides at 250,

500 and 1000 ppm concentration were tried.

The experiments were laid out with seven treatments along with control with three repetition. Completely randomized block design with factorial concept was used for analyzing the experimental data.

The percent mycelial growth inhibition obtains in each of the treatments over control were calculated using the following formula (Vincent, 1947)

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

Where,

I = Percent Inhibition
C = Colony diameter in control plate (mm)
T = Colony diameter in treated plate (mm)

Results and Discussion

Evaluation of different fungicides against *Alternaria macrospora in vitro.*

Non-systemic fungicides

All the non-systemic fungicides tested against *A. macrospora* were found effective in inhibiting the mycelial growth of *A. macrospora* as compared to control *in vitro.* As the concentration of fungicides increases, the mycelial growth inhibition also increases.

The data presented in Table 1 and Plate 1 exhibited maximum mean mycelial growth inhibition (80.53%) in copper hydroxide 53.8% DF. The next effective treatment was mancozeb 75% WP but, it was remained statistically at par with zineb 75% WP with mean mycelial growth inhibition of 75.96 and 75.26, respectively. While, minimum mean mycelial growth inhibition (18.06%) was found in wettable sulphur 80% WP.

Among the different treatments, mancozeb 75% WP and zineb 75% WP found equally effective and remained significantly superior over rest of the treatments with complete mycelial growth inhibition at 2000 ppm concentration *in vitro*. The next effective treatment in order of merit was copper hydroxide 53.8% DF with mycelial growth inhibition of 88.91 percent at 2000 ppm concentration. Propineb 70% WP remained the moderately effective treatment with 76.71 percent mycelial growth inhibition at 2000 ppm concentration. Whereas, wettable sulphur 80% WP remained the less effective as compared to other fungicides and had minimum mycelial growth inhibition of 15.54 and 17.96 percent at 500 and 1000 ppm concentration, respectively.

More or less similar kind of results were also reported by Perane *et al*. (2015) [5]. They evaluated eight fungicides against *Alternaria macrospora* and observed cent percent mycelial growth inhibition in mancozeb 75% WP followed by copper oxychloride 50% WP (75.00%) and propineb 70% WP (67.50%). Gholve *et al*. (2012) [2] also found thiram 75% WP as the most effective fungicide with 90.42 percent inhibition followed by mancozeb 75% WP (79.88%) and copper oxychloride 50% WP (71.75%). Prasad *et al*. (2018) [6] reported complete mycelial growth inhibition with mancozeb 75% WP against *A. macrospora in vitro.*

Systemic fungicides

Among different systemic fungicides, except propiconazole 25% EC and difenoconazole 25% EC found poor in inhibiting the mycelial growth of *A. macrospora* over control. As the concentration of fungicides increases, the mycelial growth inhibition also increases except propiconazole 25% EC, where inhibition remained the same in lower and higher concentration.

The data presented in Table 2 and Plate 2 indicated complete mean mycelial growth inhibition of test pathogen in propiconazole 25% EC. The next effective treatment was difenoconazole 25% EC with 84.25 percent mean mycelial growth inhibition. While, poor mean mycelial growth inhibition of 10.47 percent was observed in carbendazim 50% WP but, it was remained statistically at par with picoxystrobin 22.52% SC, thiophanate methyl 70% WP and azoxystrobin 23% SC with mean mycelial growth inhibition of 10.53, 10.77 and 11.53 percent, respectively.

Among different systemic fungicides, propiconazole 25% EC at all concentrations completely inhibited the mycelial growth and proved significantly superior over the rest of the treatments. The next effective treatment was difenoconazole 25% EC with mycelial growth inhibition of 87.23 and 84.27 percent at 500 and 250 ppm concentration, respectively. Whereas, carbendazim 50% WP found least effective treatment and recorded the minimum mycelial growth inhibition of 6.75 percent at 100 ppm concentration. But, it was remained statistically at par with picoxystrobin 22.52% SC, thiophanate methyl 70% WP, azoxystrobin 23% EC and kresoxim methyl 44.3% SC with mycelial growth inhibition of 6.98, 7.38, 7.77 and 9.14 percent at 100 ppm concentration, respectively.

The present findings are in close conformity with the results of Raut (2018) [7]. He reported propiconazole 25% EC, hexaconazole 25% EC and difenoconazole 25% EC as the most effective treatment with cent percent mycelial growth inhibition *in vitro* at 500 ppm concentration. Kapadiya *et al.* (2016) [4] also revealed complete inhibition of mycelial growth in propiconazole 25% EC followed by difenoconazole 25% EC (86.8%) at 500 ppm concentration. Perane *et al.* (2015) [5] showed 88.75 percent mycelial growth inhibition in propiconazole 25% EC.

Ready-mixed fungicides

All the ready-mixed fungicides tested against *A. macrospora* found significantly superior in inhibiting the mycelial growth of *A. macrospora* over control. As the concentration of fungicides increases, the mycelial growth inhibition also increases.

The data presented in Table 3 and Plate 3 revealed complete mean mycelial growth inhibition in fluxapyroxad 250 g/L + pyraclostrobin 250 g/L and tebuconazole 50% + trifloxystrobin 25% WG and were significantly superior over rest of the treatments. The next effective treatment was azoxystrobin 18.2% + difenoconazole 11.4% SC with 92.48 percent mycelial growth inhibition but it was remained at par with hexaconazole 4% + zineb 68% WP (92.03%). While, the minimum mean mycelial growth inhibition of 50.00 percent was recorded in copper oxychloride 50% WP + streptocycline 100% W/W.

Among the different ready-mixed fungicides, fluxapyroxad 250 g/L + pyraclostrobin 250 g/L and tebuconazole 50% + trifloxystrobin 25% WG at 250, 500 and 1000 ppm concentrations, while azoxystrobin 18.2% + difenoconazole 11.4% SC and hexaconazole 4% + zineb 68% WP at 1000 ppm concentration completely inhibited the mycelial growth

of *A. macrospora in vitro* and proved significantly superior over the rest of the treatments. Azoxystrobin 11% + tebuconazole 18.30% EC at 1000 and 500 ppm concentration and metiram 55% + pyraclostrobin 5% WG at 1000 ppm concentration found equally effective and remained the next effective treatment in inhibition of mycelial growth with 90.95, 89.82 and 89.48 percent, respectively. Copper oxychloride 50% WP + streptocycline 100% W/W revealed the least effective treatment with minimum mycelial growth inhibition of 36.29 percent at 250 ppm concentration. It is observed that the ready-mixed fungicides have given better mycelial growth inhibition than non-systemic and systemic fungicides.

The results obtained in present study is in close agreement with the results obtained by Sangeetha (2014) [8] and Raut (2018) [7]. They concluded that the hexaconazole 4% + zineb 68% WP as the most effective and showed cent percent inhibition at 1000 ppm concentration against *A. macrospora*. Indira *et al.* (2019) [3] also reported complete inhibition of mycelial growth in tebuconazole 50% + trifloxystrobin 25% EC. They reported azoxystrobin 23.5% SC + difenconazole 25% EC and azoxystrobin 23.5% SC+ tebuconazole 18.3% EC as the next effective treatment with mean mycelial growth inhibition of 94.74 and 90.94 percent respectively, against *A. macrospora in vitro*.

Table 1: Evaluation of non-systemic fungicides against *A. macrospora in vitro*

Fungicides	Mycelial growth inhibition (%)			Mean inhibition (%)
	500 ppm	1000 ppm	2000 ppm	
Chlorothalonil 75% WP	36.64 (35.61)	39.34 (40.18)	46.17 (52.04)	40.71 (42.55)
Copper hydroxide 53.8% DF	54.29 (65.93)	66.62 (84.26)	70.54 (88.91)	63.82 (80.53)
Copper oxychloride 50% WP	52.30 (62.60)	56.34 (69.28)	57.72 (71.29)	55.45 (67.84)
Mancozeb 75% WP	43.94 (48.15)	47.98 (55.19)	90.00 (100.00)	60.64 (75.96)
Propineb 70% WP	46.70 (52.96)	49.46 (57.75)	61.15 (76.71)	52.43 (62.83)
Wettable sulphur 80% WP	23.21 (15.54)	25.08 (17.96)	27.16 (20.84)	25.15 (18.06)
Zineb 75% WP	41.26 (43.49)	49.26 (57.41)	90.00 (100.00)	60.17 (75.26)
Mean	42.62 (45.85)	47.72 (54.75)	63.25 (79.74)	-
	Fungicide (F)	Concentration (C)		F x C
S. Em. ±	0.46	0.20		0.80
C. D. at 5%	1.32	0.57		2.29
C. V.%	2.71			

Note: Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values.

Table 2: Evaluation of systemic fungicides against *A. macrospora in vitro.*

Fungicides	Mycelial growth inhibition (%)			Mean inhibition (%)
	100 ppm	250 ppm	500 ppm	
Azoxystrobin 23% SC	16.18 (7.77)	20.29 (12.02)	23.08 (15.36)	19.85 (11.53)
Carbendazim 50% WP	15.06 (6.75)	18.96 (10.55)	22.62 (14.79)	18.88 (10.47)
Difenoconazole 25% EC	64.16 (81.00)	66.64 (84.27)	69.06 (87.23)	66.62 (84.25)
Kresoxim methyl 44.3% SC	17.59 (9.14)	20.68 (12.47)	25.60 (18.67)	21.29 (13.18)
Picoxystrobin 22.52% SC	15.32 (6.98)	18.75 (10.33)	22.74 (14.94)	18.94 (10.53)
Propiconazole 25% EC	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)
Thiophanate methyl 70% WP	15.76 (7.38)	19.55 (11.19)	22.18 (14.25)	19.16 (10.77)
Mean	33.44 (30.37)	36.41 (35.23)	39.32 (40.16)	-
	Fungicide (F)	**Concentration (C)**		**F x C**
S.Em. ±	0.57	0.25		0.99
C.D at 5%	1.63	0.69		2.82
C.V. %	4.71			

Note: Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values.

Table 3: Evaluation of ready-mixed fungicides against *A. macrospora in vitro.*

Fungicides	Mycelial growth inhibition (%)			Mean inhibition (%)
	250 ppm	500 ppm	1000 ppm	
Azoxystrobin 11% + Tebuconazole 18.30% EC	68.94 (87.09)	71.40 (89.82)	72.49 (90.95)	70.94 (89.34)
Azoxystrobin 18.2% + Difenoconazole 11.4% SC	64.26 (81.14)	67.99 (85.96)	90.00 (100.00)	74.08 (92.48)
Copper oxychloride 50% + Streptocycline 100% W/W	37.04 (36.29)	41.38 (43.70)	56.57 (69.65)	45.00 (50.00)
Fluxapyroxad 250 g/L + Pyraclostrobin 250 g/L SC	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)
Hexaconazole 4% + Zineb 68% WP	63.45 (80.01)	67.37 (85.19)	90.00 (100.00)	73.60 (92.03)
Metiram 55% + Pyraclostrobin 5% WG	62.31 (78.41)	66.50 (84.10)	71.08 (89.48)	66.63 (84.26)
Tebuconazole 50% + Trifloxystrobin 25% WG	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)	90.00 (100.00)
Mean	68.00 (85.97)	70.66 (89.03)	80.02 (97.00)	-
	Fungicide (F)		**Concentration (C)**	**F x C**
S. Em. ±	0.378		0.16	0.65
C. D. at 5%	1.07		0.46	1.86
C. V.%	1.55			

Note: Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values.

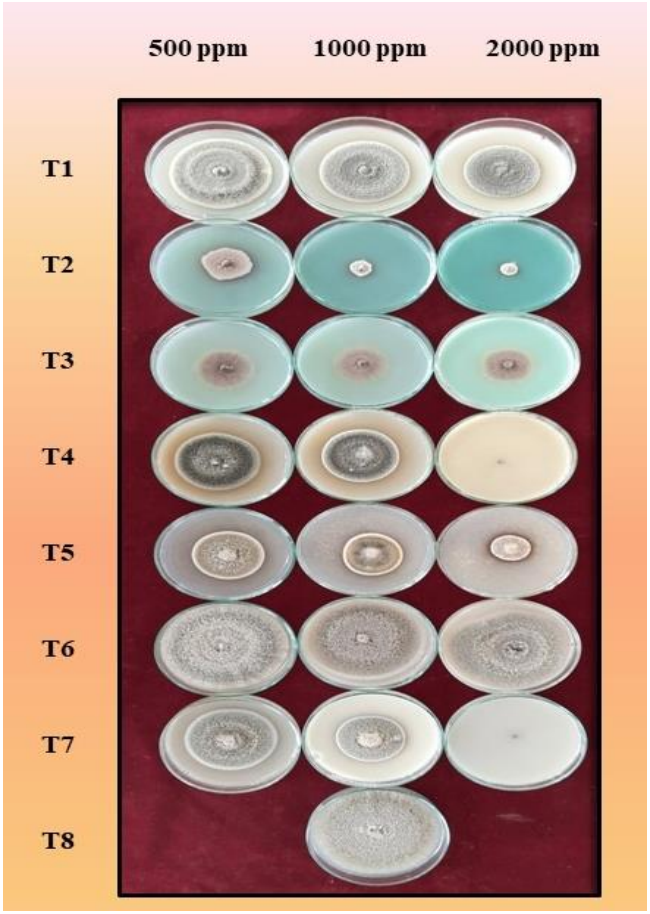

Plate 1: Evaluation of non-systemic fungicides against *A. macrospora in vitro*

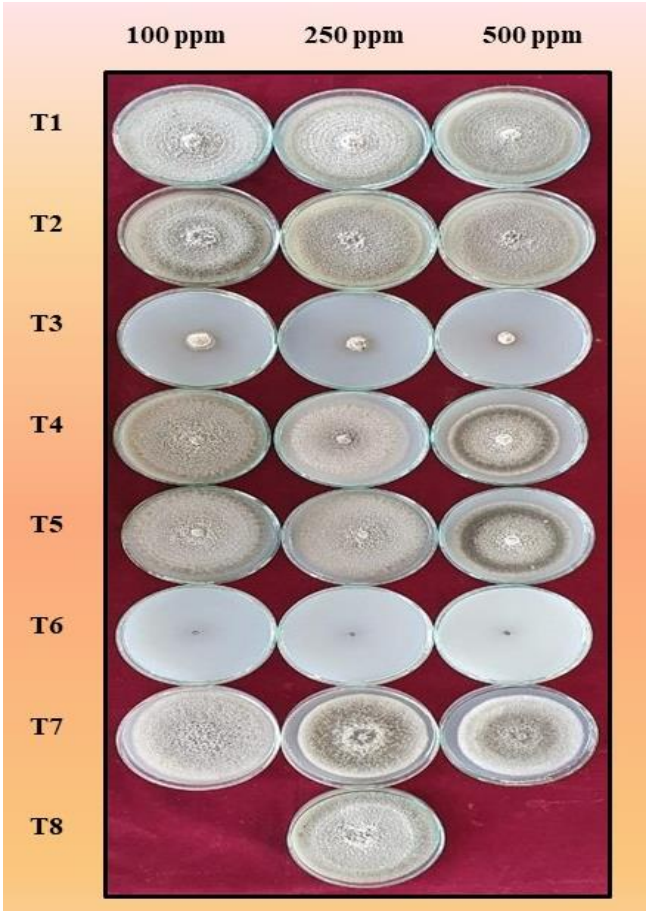

Plate 2: Evaluation of systemic fungicides against *A. macrospora in vitro*

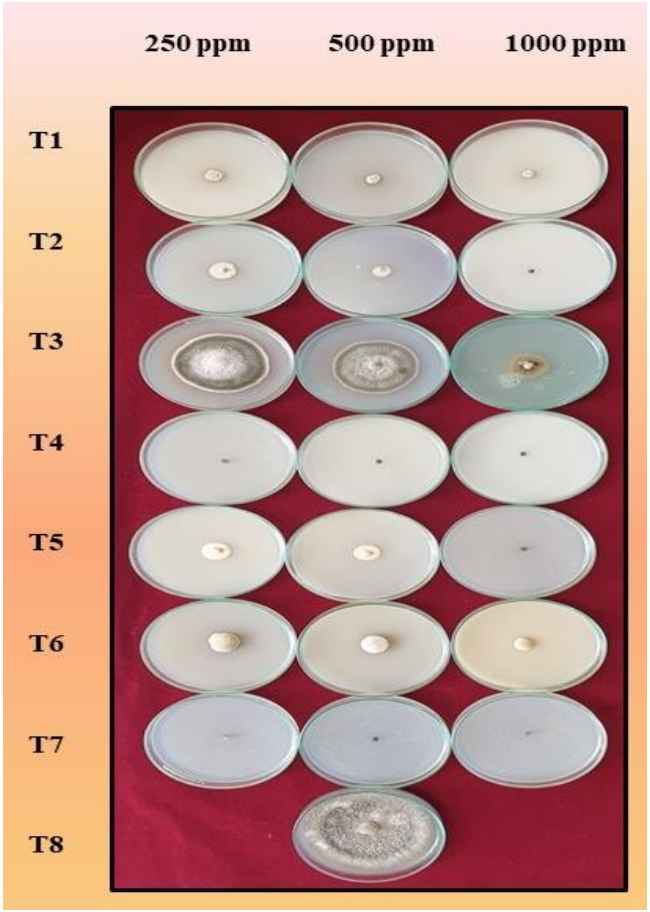

Plate 3: Evaluation of ready-mixed fungicides against *A. macrospora in vitro*

Conclusion

In order to test the efficacy of different fungicides against *A. macrospora in vitro*, different non-systemic, systemic and ready-mixed fungicides were used at different concentrations. The results revealed that, mancozeb 75% WP and zineb 75% WP among non-systemic fungicides completely inhibited the mycelial growth at 2000 ppm concentration *in vitro.* Propiconazole 25% EC among systemic fungicides completely inhibited the mycelial growth at all concentrations tried. Whereas, among ready-mixed fungicides fluxapyroxad 250 g/L + pyraclostrobin 250 g/L SC and tebuconazole 50% + trifloxystrobin 25% WG at 250, 500 and 1000 ppm concentrations and azoxystrobin 18.2% + difenoconazole 11.4% SC and hexaconazole 4% + zineb 68% WP at 1000 ppm concentration completely inhibited the mycelial growth of *A. macrospora in vitro***.**

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