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In vitro efficacy of fungicides against *Alternaria chlamydospora* causing leaf spot of okra

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Abstract
Okra (*Abelmoschus esculentus* (L.) Moench) crop is being affected by several fungal, bacterial, viral and nematode induced diseases. However, leaf spot, caused by *Alternaria chlamydospora* has been common occurrence, causing quantitative as well as qualitative losses in okra. Therefore, present *in vitro* study was planned in CRD with two separate experiments (Contact and systemic and combi-fungicides) *in vitro*, in 10 treatments replicated thrice, to assess the efficacy of fungicides against *Alternaria chlamydospora*, at the Department of Plant Pathology, College of Agriculture, Latur (MS), during 2017-18. Results revealed that All of the seven systemic and nine contact / combi-fungicides evaluated *in vitro* were found fungistatic and exhibited significant mycelial growth inhibition of *A. chlamydospora*, causing okra *Alternaria* leaf spot. However, the systemic fungicides *viz.*, Hexaconazole 5% EC, Tebuconazole 25.9% EC, Difenconazole 25% EC and Propiconazole 25% EC were found most effective against the test pathogen. Whereas, among contact / combi – fungicides the most effective fungicide found were Carbendazim 25% + Mancozeb 50% WS, Carboxin 37.5% + Thiram 37.5% WS, Tebuconazole 50% + Trifloxystrobin 25% WG, Propineb 70% WP and Mancozeb 75% WP.

Keywords: Okra, *Alternaria chlamydospora*, fungicides, inhibition

Introduction

Okra is an important vegetable crop with a diverse array of nutritional quality and potential health benefits (Gemede *et al*., 2015). India ranks first in area and production of okra and occupies an area of 507 thousands hectares under the cultivation of this crop with a production of 5853 thousands tones and productivity of 11.5 tones / ha. In Maharashtra, *Bhendi* is grown throughout the year, providing continuous and remunerative source of income to the farmers. It is extensively grown in the districts *viz*., Ahmednagar, Amravati, Aurangabad, Dhule, Jalgaon, Nagpur, Nashik, Osmanabad, Parbhani, Latur and Pune. In Maharashtra, it was cultivated on an area of 10.55 thousands hectares with an annual production of 84.50 thousands tones and productivity of 8.01 tones / ha (Anonymous, 2015).

However, biotic and abiotic stresses under changing climate are major hurdles in profitable production of various crops including okra. Okra crop is being affected by major fungal diseases *viz.*, *Alternaria* leaf spot (*Alternaria alternata / A. chlamydospora*), causing 30-50% or more yield losses (Thippeswamy *et al.*, 2007; Pansambal *et al.*, 2015) [34, 23], powdery mildew (*Erisiphe cichoracearum* and *Leveillula taurica*), causing 17-86.6% losses (Sridhar *et al.*, 1989) [29], root / charcoal rot (*Microphomina phaseolina*), wilt (*Fusarium oxysporum* f. sp. *vasinfectum*), *Cercospora* leaf spot (*Cercospora abelmoschi, C. malayensis*) and damping off (*Pythium* sp., *Rhizoctonia* sp.), viral Yellow vein mosaic (YVMV) causing 50-90% qualitative and quantitative losses (Jambhale and Nerkar, 1981) [12]. Therefore, present study on *in vitro* efficacy of fungicides against *A. chlamydospora* causing okra *Alternaria* leaf spot was planned and conducted at the Department of Plant Pathology, College of Agriculture, Latur, during 2017-18.

Materials and Methods

Isolation, identification and pathogenicity of *A. chlamydospora*

Applying tissue isolation technique (Tuite, 1969) [35], the fungus causing leaf spot disease in okra was isolated from naturally infected okra specimens, on autoclaved and cooled Potato dextrose agar (PDA) plates. Pathogenicity test attempted by spray inoculating the spore suspension (2×10^6 spores / ml) of the test pathogen, on okra seedlings in pot culture.

Based on symptomatology (naturally diseased and artificially inoculated okra plant), morpholo-cultural characteristics, microscopic observations, pathogenicity test *etc*., the test pathogen was identified as *Alternaria chlamydospora* Mouchacca and further confirmed by comparing with its authentic description (Hoog, 2000; Pansambal *et al*., 2015) [10, 23].

In *vitro* evaluation of systemic fungicides

Two separate experiments were planned to evaluated *in vitro* the efficacy of seven systemic (each @ 1000 and 1500 ppm) and five non-systemic / contact plus four combi-fungicides (each @ 2000 and 2500 ppm) and conducted with Completely Randomized Design (CRD) and all the treatments replicated thrice against *A. chlamydospora*, causing leaf spot / blight of okra by applying Poisoned Food Technique (Nene and Thapliyal, 1993) [20] and using Potato Dextrose Agar as basal culture medium. Observations on radial mycelial growth / colony diameter (mm) were in all the replicated treatments at 24 hrs interval and continued till growth of the test pathogen in untreated control plates was fully covered. Per cent inhibition of the test pathogen over untreated control was calculated by applying following formula (Vincent, 1927) [37].

$$\text{Per cent Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Growth (mm) of the test fungus in untreated control plate

T = Growth (mm) of test the fungus in treated plates

The data obtained was statistically analyzed (Panse and Sukhatme, 1978) [24] and the results were interpreted thereof.

Results and Discussion

***A. chlamydospora* inhibition**

The results obtained on per cent of mycelial growth inhibition of test pathogen *A. chlamydospora*, with the test systemic fungicides are depicted in PLATE-I and presented in Table 1 and Fig. 1. The test non systemic and combi-fungicides are depicted in PLATE-II and presented in Table 2 and Fig. 2.

Mycelial growth inhibition

Results (PLATE I, Table 1, Fig. 1) revealed that the systemic fungicides tested (each @ 1000 and 1500 ppm) significantly inhibited mycelial growth of *A. chlamydospora*, over untreated control and it was directly proportional to concentrations of the test fungicides. However, mycelial growth inhibition was cent per cent (100%) with Difenconazole 25% EC Propiconazole 25% EC, Hexaconazole 5% EC and Tebuconazole 25.9% EC at both concentrations of 1000 and 1500 ppm. These were followed by Pyraclostrobin 20% WG (72.66 and 82.22%), Thiophanate methyl 70% WP (61.77 and 71.55%) and Carbendazim 50% WP (28.22 and 34.00 %), respectively @ 1000 and 1500 ppm.

Average mycelial growth inhibition recorded with the systemic fungicides ranged from 31.11 to 100.00 per cent. However, it was significantly highest and cent per cent (100%) with Difenconazole 25% EC, Propiconazole 25% EC, Hexaconazole 5% EC and Tebuconazole 25.9% EC, followed by Pyraclostrobin 20% WG (77.44%), Thiophanate methyl 70% WP (66.66%) and Carbendazim 50% WP (31.11%).

Thus, all of the seven systemic fungicides tested were found fungistatic against *A. chlamydospora* and significantly inhibited its mycelial growth, over untreated control. However, fungicides found most effective in their order of merit were Hexaconazole 5% EC, Propiconazole 25% EC, Difenconazole 25% EC, Tebuconazole 25% WG, Pyraclostrobin 20% WG, Thiophanate methyl 70% WP and Carbendazim 50% WP.

Table 1: *In vitro* efficacy of systemic fungicide against *A. chlamydospora,* causing *Alternaria* leaf spot of okra

Tr. No.	Treatments	Colony Dia.* (mm) at ppm		Av. Colony (mm)	% Inhibition * at ppm		Av. Inhibi. (%)
		1000	1500		1000	1500	
T_1	Carbendazim 50% WP	64.60	59.40	62.00	28.22 (32.09)	34.00 (35.67)	31.11 (33.90)
T_2	Difenconazole 25% EC	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_3	Propiconazole 25% EC	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_4	Hexaconazole 5% EC	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_5	Tebuconazole 25.9% EC	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_6	Pyraclostrobin 20% WG	24.60	16.00	20.30	72.66 (58.47)	82.22 (65.06)	77.44 (61.64)
T_7	Thiophanate methyl 70% WP	34.40	25.60	30.00	61.77 (51.81)	71.55 (57.77)	66.66 (54.73)
T_8	Control	90.00	90.00	90.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
	S.E. $\pm$	0.27	0.30	0.33	0.27	0.25	0.33
	C.D. (P=0.01)	0.79	0.89	0.98	0.79	0.83	0.98

*: Mean of three replications, Dia: Diameter, Av.: Average, Inhibi: Inhibition

Figures in parentheses are arcsine transformed value

These results are in conformity to the reports of several earlier workers. Fungicides Hexaconazole 5% EC, Tebuconazole 25% WG, Difenconazole 25% EC, Propiconazole 25% EC and Pyraclostrobin 20 % WG were reported to cause maximum mycelial growth inhibition in many *Alternaria* spp. (Mesta *et al.*, 2007; Raja and Reddy, 2008; Patel and Chaudhari, 2010; Naik *et al.*, 2010; Thaware *et al.*, 2010; Yadav *et al*., 2013a; Apet *et al*., 2014; Harde and Atar, 2014; Joshi *et al*., 2014; Waghe *et al*., 2014; Pansambal *et al*., 2015; Prasanna, 2016; Jakatimath *et al*., 2017; Kumar *et al*., 2017; Kumar and Singh, 2017; Jhala and Mali, 2017;\ Vijayalakshmi *et al*., 2018) [28, 25, 19, 33, 39, 2, 9, 14, 38, 23, 27, 11, 16, 17, 13, 36]

Mycelial growth inhibition

Results (Table 2, Fig.2) revealed that non-systemic / contact and combi- fungicides tested (each @ 2000 and 2500 ppm), significantly inhibited mycelial growth of *A. chlamydospora* in the range of 24.44 to 100 per cent, respectively @ 2000 and 2500 ppm over untreated control and it was directly proportional to the fungicides tested.

The fungicides Carbendazim 25% + Mancozeb 50%, Carboxin 37.5% + Thiram 37.5%, Tebuconazole 50%

+Trifloxystrobin 25% and Carbendazim 25% + Mancozeb 64% WP @ 2000 and 2500 ppm and Mancozeb 75% WP, Propineb 70% WP, Cymoxanil 8% + Mancozeb 64% WP @ 2500 ppm resulted in cent per cent (100%) mycelial growth inhibition. These were followed by Copper hydroxide 77% WP (73.44% and 75.00%), Copper oxychloride 50% WP (56.66% and 62.44%) respectively @ 2000 and 2500 ppm. However, Chlorothalonil 75% WP was found least effective.

Table 2: *In vitro* bioefficacy of non-systemic and combi-fungicides against *A. chlamydospora,* causing okra leaf spot

Tr. No	Treatments	Colony Dia.* (mm) at ppm		Av. (mm)	% Inhibition * at ppm over control		Av. Inhibition (%)
		2000	2500		2000	2500	
T_1	Mancozeb 75% WP	30.00	00.00	15.00	66.66 (54.73)	100.00 (90.00)	83.33 (35.26)
T_2	Copper oxychloride 50% WP	39.00	33.80	36.40	56.66 (48.83)	62.44 (52.20)	59.55 (50.51)
T_3	Propineb 70% WP	40.80	00.00	20.40	54.66 (47.67)	100.00 (90.00)	77.33 (61.57)
T_4	Chlorothalonil 75% WP	68.00	63.60	65.80	24.44 (29.63)	29.33 (32.79)	26.89 (31.24)
T_5	Copper hydroxide 77% WP	23.90	22.50	23.20	73.44 (58.98)	75.00 (60.00)	74.22 (59.49)
T_6	Carboxin 37.5% + Thiram 37.5% (75% DS)	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_7	Cymoxanil 8% + Mancozeb 64% (72% WP)	05.60	00.00	02.80	93.77 (75.55)	100.00 (90.00)	96.89 (89.84)
T_8	Tebuconazole 50% +Trifloxystrobin 25% (75% WG)	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_9	Carbendazim 25% + Mancozeb 50% (75% WS)	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_{10}	Control (Untreated)	90.00	90.00	90.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
	S.E. ±	0.54	0.73	0.77	0.83	0.70	0.36
	C.D. (P=0.01)	1.66	2.22	2.33	2.46	2.20	1.38

*: Mean of three replications, Dia: Diameter, Av.: Average
Figures in parentheses are arcsine transformed value

Average mycelial growth inhibition with the test fungicides ranged from 26.89 to 100 per cent. However, it was cent per cent (100%) with three fungicides *viz.*, Carbendazim 25% + Mancozeb 50%, Carboxin 37.5% + Thiram 37.5%, Tebuconazole 50% +Trifloxystrobin 25%. These were followed by Cymoxanil 8% + Mancozeb 64% (96.89%), Mancozeb 75% WP (83.33%), Propineb 70% WP (77.33%), Copper hydroxide 77% WP (74.22%), Copper oxychloride 50% WP (59.55%).

Thus, on the basis of efficiency, fungicides in the order of merit were Carbendazim 25% + Mancozeb 50%, Carboxin 37.5% + Thiram 37.5% > Tebuconazole 50% + Trifloxystrobin 25% > Cymoxanil 8% + Mancozeb 64% > Mancozeb 75% WP > Propineb 70% WP > Copper hydroxide 77% WP and Copper oxychloride 50% WP.

These results of the present study are in harmony concedes with the findings of many earlier workers, who reported effectiveness of non-systemic / contact fungicides such as Propineb, Mancozeb, Copper oxychloride, Chlorothalonil *etc.* against many phytopathogenic *Alternaria* spp. (Singh and Singh, 2006; Kadam and Deshmukh, 2010; Chaudary, 2010; Chetana *et al*., 2012; Ganie *et al.*, 2013b; Nikam *et al.*, 20014) [28, 25, 3, 6]; combi-fungicides *viz.*, Carboxin 37.5% + Thiram 37.5%, Tebuconazole 50% + Trifloxystrobin 25%, Carbendazim 25% + Mancozeb 50%, Cymoxanil 8% + Mancozeb 64%, etc. against many phytopathogenic *Alternaria* spp. (Singh and Singh, 2006; Deshmukh *et al*., 2008; Raja and Reddy, 2008; Naik *et al.*, 2010; Thaware *et al.*, 2010; Yadav *et al*., 2013a; Apet *et al*., 2014; Gholve *et al.*, 2014; Harde and Atar, 2014; Joshi, *et al.*, 2014; Pansambal *et al*., 2015; Kumar and Burnwal, 2016; Prasanna, 2016; Jakatimath, *et al*., 2017; Kumar *et al*., 2017; Kumar and Singh, 2017; Prasad *et al.*, 2018; Jhala and Mali, 2017; Vijayalakshmi *et al*., 2018) [28, 25, 19, 33, 39, 2, 9, 14, 38, 23, 27, 11, 16, 17, 13, 36].

Conclusion

Hence, from ongoing results and discussion, it is concluded that all of the seven systemic (each @ 1000 and 1500 ppm) and nine contact and combi-fungicides (each @ 2000 and 2500 ppm) evaluated *in vitro* were found effective and fungistatic against *A. chlamydospora*. However, Based on average mycelial growth inhibition, the systemic fungicides *viz.*, Hexaconazole 5% EC, Tebuconazole 25.9% EC, Difenconazole 25% EC and Propiconazole 25% EC were found most effective against the test pathogen. Whereas, among contact / combi – fungicides the most effective fungicide found were Carbendazim 25% + Mancozeb 50% WS, Carboxin 37.5% + Thiram 37.5% WS, Tebuconazole 50% + Trifloxystrobin 25% WG, Propineb 70% WP and Mancozeb 75% WP.

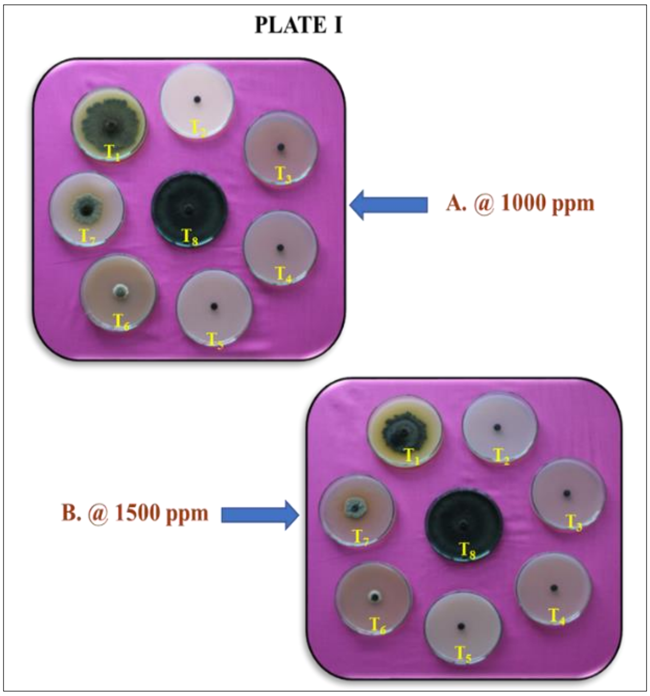

Plate I: *In vitro* efficacy of systemic fungicides against *A. chlamydospora*

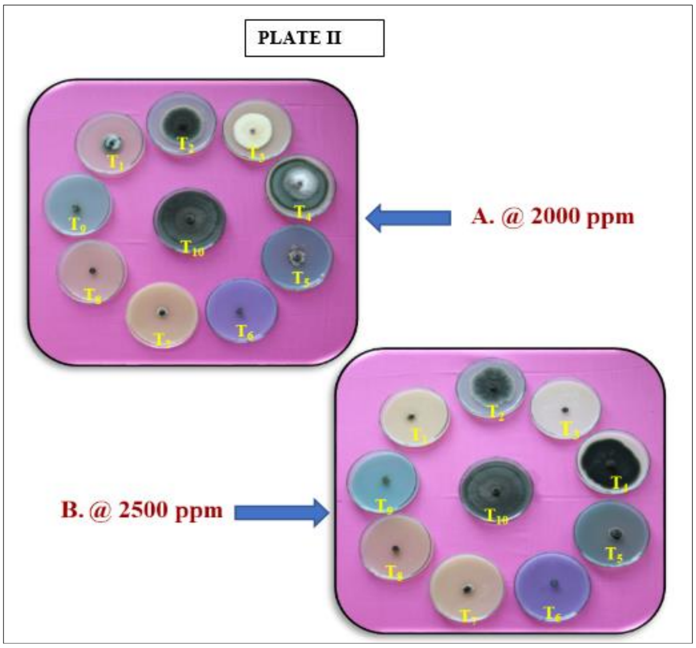

Plate II: *In vitro* efficacy of contact and combi-fungicides against *A. chlamydospora,* causing okra leaf spot

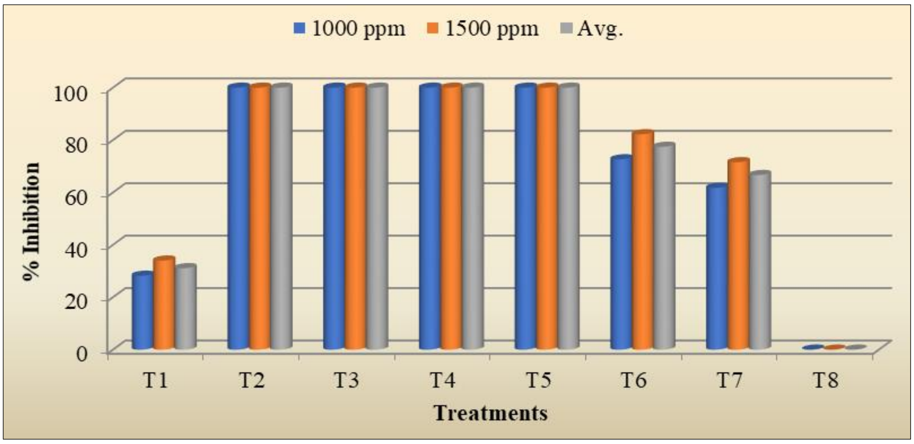

Fig 1: *In vitro* efficacy of systemic fungicides against *A. chlamydospora,* causing okra leaf spot

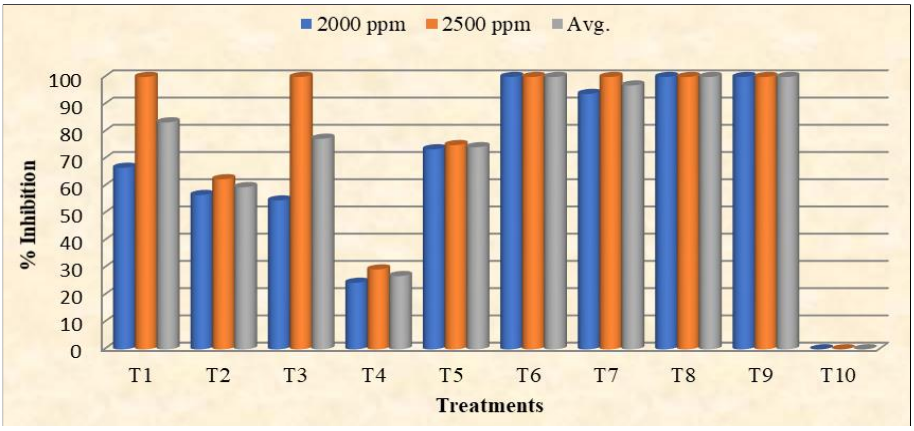

Fig 2: *In vitro* efficacy of contact and combi-fungicides against *A. chlamydospora,* causing okra leaf spot

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