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In vitro evaluation of different fungicides against *Alternaria macrospora* causing leaf spot of cotton

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Abstract

A study has been conducted at Department of Plant Pathology, University of Agricultural Sciences Raichur on evaluation of new molecules of fungicides against *Alternaria macrospora*, causing leaf spot of cotton. Seven systemic, eight non systemic and combi fungicides, respectively were evaluated against *A. macrospora* under *in vitro* by poison food technique. Among the systemic fungicides evaluated, difenconazole, hexaconazole, propiconazole and tebuconazole at all the concentrations *viz.*, 0.05, 0.1 and 0.2 per cent recorded complete inhibition (100%) of mycelial growth of *A. macrospora*. Among the non systemic fungicides, mancozeb was found most effective and recorded significantly highest mean mycelial inhibition (90.10%). Among the combi fungicides evaluated, trifloxystrobin 25% + tebuconazole 50% EC and captan 70% + hexaconazole 5% WP were found most effective and recorded complete inhibition of mycelial growth of *A. macrospora* at all the three concentrations (0.1, 0.2 and 0.3%).

Keywords: Alternaria macrospora, systemic fungicides, non systemic fungicides, combi fungicides

Introduction

Cotton (*Gossypium* spp.) is one of the most important commercial crop which plays a vital role in the national economy. It is popularly called as 'white gold' and 'king of fibre'. India occupies first place in area and second place in *Bt* cotton production after china with the production of 351 lakh bales of 170 kg lint from an area of 105 lakh ha with productivity of 568 kg⁻¹ ha (Anon., 2017)^[2]. In Karnataka, *Bt* cotton occupies an area of 6.33 lakh ha with a production of 18.02 lakh bales with a productivity of 510 kg⁻¹ ha (Anon., 2015)^[1]. Among the various diseases infecting *Bt* cotton, *Alternaria* leaf spot caused by *Alternaria macrospora* Zimm. is considered as a potentially destructive disease in Karnataka and is reported to cause the yield loss of 5-35 per cent (Zanjare *et al.*, 2005 and More *et al.*, 2010)^[15, 7]. There is no resistant variety or hybrid available against this disease. Thus it has become inevitable to go for fungicidal sprays for management of disease. In the present study some of the new chemicals were tested in the laboratory to find out their efficacy against *A. macrospora*.

Materials and Methods

The study has been conducted on evaluation of new molecules of systemic, contact and combi fungicides against *A. macrospora* in the Department of Plant Pathology, University of Agricultural Sciences Raichur, during 2017-18. Efficacy of seven systemic fungicides were evaluated at 0.05, 0.1 and 0.2 per cent concentrations. Whereas, non systemic and combi fungicides were evaluated at 0.1, 0.2 and 0.3 per concentrations using PDA as a basal medium by poisoned food technique (Nene and Thapliyal, 1993)^[8].

The required quantities of individual fungicides were added separately into molten and cooled potato dextrose agar to get the desired concentration of the fungicides. Later 20 ml of such poisoned medium was poured into sterile Petri plates. Mycelial discs of 5 mm size from actively growing culture of the test fungus was cut out by a sterile cork borer and one such disc was placed at the centre of each agar plate. Control was maintained without adding any fungicides to the medium and each treatment was replicated thrice. Then such plates were incubated at room temperature for 12 days and radial colony growth (mm) was measured. The efficacy of a fungicide was expressed as per cent inhibition of mycelial growth over control that was calculated by the following formula suggested by Vincent (1947)^[14].

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

Where,

$$\begin{split} I &= \text{Per cent inhibition} \\ C &= \text{Radial growth of fungus in control} \\ T &= \text{Radial growth of fungus in treatment} \end{split}$$

Statistical analysis

Statistical analysis was carried out as per the procedures given by Panse and Sukhathme (1985)^[9]. Actual data in percentage were converted to arc sine values, before analysis according to the table given by Snedecor and Cochran (1967)^[13].

Results and discussion

Efficacy of systemic fungicides against A. macrospora of cotton

Efficacy of seven systemic fungicides viz., carbendazim, difenconazole, hexaconazole, propiconazole, tebuconazole, pyraclostrobin and azoxystrobin was tested against A. macrospora at different concentrations (0.05, 0.1 and 0.2%) by poisioned food technique. Among the systemic fungicides, difenconazole, hexaconazole, propiconazole and tebuconazole were highly effective in inhibiting the radial growth (100%) at all the concentrations tested under in vitro, followed by pyraclostrobin and azoxystrobin with mean mycelial inhibition of 86.02 and 63.72 per cent respectively. The interaction between fungicides and concentrations on inhibition of mycelial growth was found significant (Table 1). Similar results were found in investigations made by Gangurde et al. (2004)^[5], Ramegouda (2007)^[11], Dighule et al. (2011)^[3] and Sangeetha and Ashtaputre (2013)^[12] where triazole fungicides were proven to show good inhibition on growth of A. macrospora. The triazole group of fungicides are the potent inhibitors of 'ergosterol biosynthesis', the major membrane sterol of fungi and they also block the cytochrome P450-dependent enzyme C-14 alpha-demethylase, which is needed to convert lanosterol to ergosterol. Carbendazim showed least effect (18.72%) against A. macrospora as it is not effective against coloured spore producing fungus (Rajpurohit et al., 1984) ^[10]. The systemic fungicides belonging to strobulirin group i.e., azoxystrobin 23.5% SC, pyraclostrobin 50% WG have not shown much efficacy in control of growth of A. macrospora. The interaction between fungicides and concentrations on inhibition of mycelial growth was found significant. The results were in contradiction with the studies made by Ganeshan and Chetana (2009)^[4], where strobulin group of fungicides are found least effective in control of Alternaria spp.

Efficacy of non-systemic fungicides against A. macrospora of cotton

Efficacy of eight non-systemic fungicides viz., carbendazim, difenconazole, hexaconazole, propiconazole, tebuconazole, pyraclostrobin and azoxystrobin was tested against A. macrospora at different concentrations (0.05, 0.1 and 0.2%) by poisioned food technique. The results revealed that there was a significant difference between the treatments with respect to per cent mycelial inhibition of mycelial growth. Among the eight contact fungicides tested, mancozeb was found most effective which recorded maximum growth inhibition of 85.17, 90.50 and 94.62 per cent at 0.1, 0.2 and 0.3 per cent concentrations respectively, with significantly highest mean growth inhibition (90.10%) of A. macrospora (Table 2). Mancozeb belongs to dithiocarbamate fungicide group which has the broad spectrum and multi-site actions in fungi like, it inhibits the process of respiration and RNA synthesis process in fungi. The efficacy of dithiocarbamate fungicide on different Alternaria spp. has confirmed by the investigations carried out by (Glovle et al., 2012). The next best were thiram and captan which recorded mean mycelial inhibition of 89.37 and 81.94, respectively. The other fungicides were also found effective viz., propineb (80.26%) and copper oxychloride (72.08%). Least inhibition was recorded by zineb (36.89%).

Efficacy of non-systemic fungicides against A. macrospora of cotton

Development of resistance in many pathogens to fungicides with single point action has lead way to the development of new fungicides where chemicals with two different mode of action, which showed synergistic effect for the control of pathogens. So an effort made to evaluate different combi fungicides against A. macrspora. The results revealed that all the combi fungicides tested inhibited the growth of A. macrospora at various concentrations and the effect was significant (Table 3). Irrespective of the concentrations at the eight day maximum inhibition of mycelial growth over the control was recorded in trifloxystrobin 25% + tebuconazole 50% EC and captan 70% + hexaconazole 5% WP (100.00%) which were significantly superior over other treatments and were found to be the most effective chemicals. The next best were azoxystrobin 23.5% SC + difenconazole 25% EC, cymanaxil 8% + mancozeb 64% WP and azoxystrobin 23.5% SC+ tebuconazole 18.3% EC with mean mycelial inhibition of 94.74, 92.63 and 90.94 per cent respectively. While, carbendazim 12% + mancozeb 63% WP has proven to be least effective with an inhibition of 65.74 per cent. Result obtained in present the study on antifungal effects of the test fungicides against A. macrospora are in consonance with findings of Dighule et al. (2011)^[3].

 Table 1: In vitro evaluation of systemic fungicides on mycelial growth of Alternaria macrospora.

	Fungicides	Per cent inhibition Concentrations (%)			
Tr. No.					Mean
		0.05	0.1	0.2	
T1	Azoxystrobin	36.86* (37.39)**	69.41 (56.43)	84.90 (67.14)	63.72 (52.97)
T2	Carbendazim	13.43 (21.50)	18.90 (25.77)	23.85 (29.24)	18.72 (25.64)
T3	Difenconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T4	Hexaconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T5	Propiconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T6	Pyraclostrobin	83.09 (65.72)	85.56 (67.67)	89.41 (71.01)	86.02 (68.05)
T7	Tebuconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	Mean	76.19 (60.80)	81.98 (64.89)	85.45 (67.58)	
			S. Em. ±		CD at 1%

1. Fungicides (F)	0.39	1.23
2. Concentrations (C)	0.26	0.84
3. $F \times C$	0.69	1.97

* Original value ** Arc sine transformed value

Table 2: In vitro evaluation of non systemic fungicides on mycelial growth of A. macrospora

		1			
T. No	Fungicides	Concentrations (%)			Maan
11. NO.					Iviean
		0.1	0.2	0.3	
T1	Captan	77.50* (61.69)**	81.83 (64.78)	86.50 (68.45)	81.94 (64.86)
T2	Chlorothalonil	18.83 (25.73)	32.33 (34.66)	62.00 (51.95)	37.72 (39.70)
T3	Copper oxy chloride	51.76 (46.13)	77.67 (61.92)	86.83 (68.73)	72.08 (58.11)
T4	Copper hydroxide	30.67 (33.63)	51.96 (46.13)	77.84 (61.92)	53.49 (47.01)
T5	Mancozeb	85.17 (67.35)	90.50 (72.05)	94.62 (76.59)	90.10 (71.67)
T6	Propineb	65.88 (54.27)	80.20 (63.58)	94.71 (76.70)	80.26 (63.63)
T7	Thiram	84.33 (66.69)	89.61 (72.10)	94.17 (76.03)	89.37 (70.98)
T8	Zineb	16.67 (24.10) 28.84 (32.10)		65.17 (53.83)	36.89 (37.40)
	Mean	61.54 (51.68)	76.13 (60.75)	94.47 (76.40)	
			S. Em. ±		CD at 1%
	1. Fungicides (F)		0.42		1.20
	2. Concentrations (C)		0.25		0.73
	3. F >	< C	0.73		2.17

* Original value ** Arc sine transformed value

Table 3: Efficacy of combi fungicides on inhibition of mycelial growth of A. macrospora under in vitro conditions.

		Per cent inhibition				
Tr. No.	Fungicide	Concentrations (%)			Mean	
		0.1	0.2	0.3		
T1	Azoxystrobin 23.5%SC + difenconazole25% EC	93.33* (75.04)**	94.83 (76.87)	96.08 (78.59)	94.74 (76.75)	
T2	Cymanaxil 8% + mancozeb 64% WP	90.58 (72.13)	92.00 (73.58)	95.33 (77.53)	92.63 (74.25)	
T3	Pyraclostrobin 5% + metiram 55.5% WP	81.32 (64.40)	85.23 (67.40)	88.42 (70.11)	84.99 (67.21)	
T4	Azoxystrobin 11% + tebuconazole 18.3%	88.56 (70.24)	90.77 (72.32)	93.50 (75.32)	90.94 (72.49)	
T5	Tricyclozole 18% + mancozeb 62%WP	62.40 (52.19)	75.00 (60.00)	78.37 (62.29)	71.92 (58.01)	
T6	Trifloxystrobin 25% + tebuconazole 50%EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	
T7	Carbendazim 12% + mancozeb 63%WP	63.63 (52.91)	65.19 (53.85)	68.40 (55.80)	65.74 (54.18)	
T8	Captan 70% + hexaconazole 5% WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	
	Mean	84.97 (67.19)	87.87 (69.62)	90.01 (71.58)		
				S. Em. ±	CD at 1%	
	1. Fungicides (F)			0.28	0.79	
	2. Concentrations (C)			0.17	0.48	
	$3. F \times C$			0.48	1.38	

* Original value ** Arc sine transformed value

Conclusion

In vitro evaluation of different fungicides revealed that, among systemic fungicides difenconazole, hexaconazole, propiconazole and tebuconazole were highly effective in inhibiting the radial growth (100%) at all the concentrations (0.05, 0.1 and 0.2%). Among the non systemic fungicides, mancozeb was found most effective which recorded significantly highest mean growth inhibition (90.10%) and among the combi fungicides, trifloxystrobin 25% + tebuconazole 50% EC and captan 70% + hexaconazole 5% WP (100.00%) were significantly superior over other treatments and were found to be the most effective chemicals.

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