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In vitro evaluation of fungicides against *Alternaria* leaf spot of cotton

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Abstract
Cotton (*Gossypium hirsutum* L.) is the one of the most important commercial crops of the world, which belongs to the family Malvaceae. India is the largest cotton growing country in the world with an area of around 12.35 M ha followed by United States and China with production of 36.1 M bales and productivity of 524 kg lint/ha. Cotton crop is known to suffer from number of fungal, bacterial and viral diseases. Cotton is under constant threat of foliar diseases *viz.*, grey mildew, *Alternaria* leaf spot, *Myrothecium* leaf spot, bacterial leaf blight, rust etc. Among them, *Alternaria* leaf spot caused by *Alternaria* spp. is predominant in causing economic losses to the cotton crop in the country. Evaluation of systemic, non-systemic and combined fungicides under *in vitro* conditions revealed that the among eight fungicides evaluated against *Alternaria macrospora* (N2A isolate), Propiconazole, Hexaconazole and Tebuconazole @ 500 and 1000 ppm recorded maximum inhibition with 100 per cent inhibition and no radial growth of mycelium was observed. Pyraclostrobin and Azoxystrobin @1000 ppm showed 91.0 and 87.2 per cent inhibition respectively. Carbendazim+Mancozeb (SAAF) @ 1500 ppm and 2000 ppm showed 96.1 and 96.9 per cent inhibition respectively.

Keywords: Fungicides, *Alternaria*, leaf spot, cotton

Introduction
Cotton is the most essential natural fiber crop in the world for textile produce, accounting for about 50% of all fibers used in the textile industry. It is grown all over the world in about 80 countries. Cotton is unique among agricultural crops, because it is the main natural fiber crop, and also provides edible oil. It is one of the agro-industrial crops which are produced in both developing and developed countries (Bedane and Arkebe, 2019) [2].

India is the largest cotton growing country in the world with an area of around 12.35 M ha followed by United States and China with production of 36.1 M bales and productivity of 524 kg lint/ha (Cotton Association of India, 2018-2019). India's share in global cotton exports is around 25 per cent In India, Maharastra (26.63%), Gujarat (17.96%), Andhra Pradesh (13.75%) and also Madhya Pradesh are the leading cotton producing states. Cotton in India provides direct livelihood to 6 million farmers and about 40 -50 million people are employed in cotton trade and its processing (Chitte *et al.*, 2019) [4].

The word "cotton" is derived from Arabic word (*qutn* or *qutun*). This was the usual word for cotton in medieval Arabic. Cotton (*Gossypium* spp.) belongs to the botanical family Malvaceae. Cotton is referred to as "King of Fibres" and also known as "White Gold" (Prasad *et al.*, 2018) [8].

There are four cultivated species of cotton *viz.*, *Gossypium arboreum, G. herbaceum, G. hirsutum* and *G. barbadense*. The first two species are diploid (2n=26) and are native to old world. The last two species are tetraploid (2n=52). *G. hirsutum* is the predominant species which alone contributes about 90 per cent to the global production. Perhaps, India is the only country in the world where all the four cultivated species are grown on commercial scale (Chitte *et al.*, 2019) [4].

The various species of cotton grown as agricultural crops are native to subtropical parts of the world. Cotton can be found as perennial tree like plants in tropical climate but is normally cultivated as a shrubby annual in temperate climates.

Cotton is a heat loving crop. During germination it requires 32-34 ºC and 25-27 ºC during the vegetative stage. Average temperature of 21-22 ºC is required for the crop (Sangameshwari *et al.*, 2019) [10]. It is grown between latitudes 30° N and 30° S.

Materials and Methods

Selected fungicides were evaluated to study the effect of fungicides against *Alternaria* sp. under *in vitro* conditions by employing poisoned food technique (Nene and Thapliyal, 1993). Ten ml of stock solution of 10 per cent concentration is prepared in sterile distilled water. Twenty ml of poisoned medium is poured into sterilized Petri plates under aseptic conditions in the laminar air flow chamber and allowed to solidify. These plates were inoculated with 5 mm diameter disc cut from actively growing periphery regions of *Alternaria* sp. culture and incubated at 28 ± 1 ºC. PDA plates containing non poisoned food medium and inoculated with *Alternaria* sp. serves as control. Radial growth of pathogen and per cent inhibition is calculated by using the formula.

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

Where, I = Per cent inhibition over control
C = Radial growth of pathogen in control (mm)
T = Radial growth of pathogen in treatment (mm)

List of fungicides evaluated against *Alternaria* sp:

Treatment	Chemical name	Trade name	Rate of concentration
T1	Hexaconazole	Contaf	500ppm, 1000ppm
T2	Tebuconazole	Folicur	500ppm, 1000ppm
T3	Propiconazole	Tilt	500ppm, 1000ppm
T4	Pyraclostrobin	Headline	500ppm, 1000ppm
T5	Azoxystrobin	Amistar	500ppm, 1000ppm
T6	Carbendazim12 %+Mancozeb 63%	SAAF	1500ppm, 2000ppm
T7	Mancozeb	Indofil	1500ppm, 2000ppm
T8	Copper oxychloride	Blitox	1500ppm, 2000ppm

Results

Evaluation of systemic fungicides against *Alternaria macrospora* under *in vitro* conditions

Five systemic fungicides *viz.*, Propiconazole, Hexaconazole, Tebuconazole, Pyraclostrobin and Azoxystrobin were tested *in vitro* against *Alternaria* spp. using poisoned food technique as described in material and methods. Isolate N2A was selected as the test pathogen since it was morphologically similar to A. *macrospora* and also showed maximum resemblance in molecular identification method. All five systemic fungicides evaluated *in vitro* were significantly found to influence mycelial growth and its corresponding inhibition of *Alternaria* spp. at concentrations each @ 500 and 1000 ppm (Table.1, Figure.1 and Plate.1). Mycelial growth and its inhibition were found inversely and directly proportional, respectively to concentrations of the fungicides tested. Propiconazole, Hexaconazole and Tebuconazole @ 500 and 1000 ppm showed complete inhibition with no radial growth of mycelium whereas, the radial growth in Pyraclostrobin and Azoxystrobin @ 500 was 11.50 mm and 16.83 mm with 87.2 and 81.3 per cent inhibition respectively. Pyraclostrobin and Azoxystrobin @ 1000 ppm treatment recorded 8.0 mm and 11.50 mm radial growth respectively and its per cent inhibition was 91.0 and 87.2 per cent respectively.

Table 1: Evaluation of systemic fungicides against *Alternaria macrospora* under *in vitro* conditions

Treatment No.	Treatments	Colony diameter (mm) at ppm		Per cent inhibition at ppm	
		500	1000	500	1000
1	Propiconazole	00.00	00.00	100	100
				(90.00)	(90.00)
2	Hexaconazole	00.00	00.00	100	100
				(90.00)	(90.00)
3	Azoxystrobin	16.83	11.50	81.3	87.2
				(63.48)	(68.61)
4	Tebuconazole	00.00	00.00	100	100
				(90.00)	(90.00)
5	Pyraclostrobin	11.50	8.00	87.2	91.1
				(68.61)	(72.56)
	Control	90.00	90.00	00.00	00.00
				(00.00)	(00.00)
	C.D.	0.752	0.583	0.512	0.412
	SE(m) ±	0.236	0.183	0.227	0.129
	C.V.	7.204	8.108	0.296	0.234

*Average of three replications.
Figures in parentheses indicate angular transformed value.

Evaluation of non-systemic and combined fungicides agains *Alternaria macrospora* under *in vitro* conditions

Two non-systemic fungicides *viz.*, Mancozeb and Copper oxychloride @ 1500 and 2000 ppm were evaluated under *in vitro* conditions using poisoned food technique as described in 3.8. The radial growth of Mancozeb and Copper oxychloride @ 1500 ppm observed was 14.50 and 12.50 mm respectively with per cent inhibition of 83.8 and 86.1 per cent respectively. While, the radial growth of Mancozeb and Copper oxychloride @ 2000 ppm observed was 12.5 mm and 6.46 mm respectively and showed 86.1 and 92.8 per cent inhibition respectively. Carbendazim+Mancozeb recorded the radial growth of 3.43 mm and
96.1 per cent inhibition at 1500 ppm whereas, radial growth @ 2000 ppm was 2.76 with per cent inhibition of 96.9 per cent, which is significantly superior than non-systemic fungicide (Table. 2, Figure.2 and Plate.2)

Table 2: Evaluation of non-systemic and combi fungicides against *Alternaria macrospora* under *in vitro* conditions

Treatment No.	Treatments	Colony diameter (mm) at ppm		Per cent inhibition at ppm	
		1500	2000	1500	2000
1	Mancozeb	14.50	12.5	83.8	86.1
				(65.57)	(67.95)
2	Copper oxychloride	12.50	6.46	86.1	92.8
				(67.89)	(73.26)
3	Carbendazim12%+Mancozeb 63%	3.43	2.76	96.1	96.9
				(78.21)	(79.61)
	Control	90.00	90.00	00.00	00.00
				(00.00)	(00.00)
	C.D.	0.958	1.214	0.650	0.986
	SE(m) ±	0.271	0.344	0.184	0.279
	C.V.	4.635	8.231	0.360	0.528

*Average of three replications.
Figures in parentheses indicate angular transformed value

The result of present studies are in agreement with the findings of *viz.*, Arun Kumar (2008) [1] who reported that out of nine different fungicides tested *in vitro*, Propiconazole, Hexaconazole at all the concentration (0.1%, 0.2% and 0.3%) completely inhibited the mycelial growth of A. *alternata* infecting cotton.

Thaware *et al.* (2010) [11] evaluated different fungicides under *in vitro* conditions against leaf blight of cotton caused by A. *alternata* and showed that Mancozeb @ 0.2 per cent and Propiconazole@ 0.05 per cent completely inhibited the mycelial growth of test fungus.

Berman *et al.* (2015) [3] estimated different fungicides against leaf blight of tomato caused by A. *alternata* @ 0.2 per cent concentration. They concluded that Bavistin showed 100 per cent inhibition followed by Captaf (81.2%), Nystatin (73%).

Mohan *et al.* (2018) [6] evaluated eleven fungicides against leaf blight of cotton caused by *A. macrospora* under *in vitro* conditions. Among all the tested fungicides mancozeb, carbendazim, hexaconazole, propiconazole and carbendazim + mancozeb showed complete inhibition (100%) at all the tested dosages followed by captan + hexaconazole (89.74%), thiram and captan (78.89%) and azoxystrobin showed least inhibition (56.81%) compared to untreated control.

Raut *et al.* (2019) [9] evaluated seven non systemic fungicides *viz.*, Chlorothaonil 75WP, Propineb 70WP, Mancozeb 75WP, Copper oxychloride 50WP, Captan 50WP, Thiram 75WP and Ziram 27EC each @ 1500 and 2000 ppm concentration against A. *macrospora* which causes leaf blight of cotton. Among eleven fungicides maximum per cent inhibition was observed with Mancozeb (86.95%), followed by Thiram (84.68%), Ziram (81.76%), Propineb (80.23%) and Copper oxychloride (75.57%), whereas, it was comparatively minimum with Chlorothaonil (55.29%) and Captan (73.10%).

Discussions

Evaluation of systemic, non-systemic and combined fungicides against *Alternaria macrospora* (N2A isolate) under *in vitro* conditions revealed that Propiconazole, Hexaconazole and Tebuconazole showed maximum inhibition (100%) @ 500 and 1000 ppm and no radial growth of mycelium was observed. Pyraclostrobin and Azoxystrobin at 500 ppm recorded 87.2 and 81.3 per cent inhibition respectively, whereas @1000 ppm it was 91.0 and 87.2 per cent inhibition respectively. Mancozeb and Copper oxychloride@ 1500 ppm recorded 83.8 and 86.1 per cent inhibition respectively and it was 86.1 and 92.8 per cent at 2000 ppm respectively. Combined fungicide Carbendazim+Mancozeb (SAAF) @ 1500 ppm and 2000 ppm inhibited to the extent of 96.1 per cent and 96.9 per cent respectively.

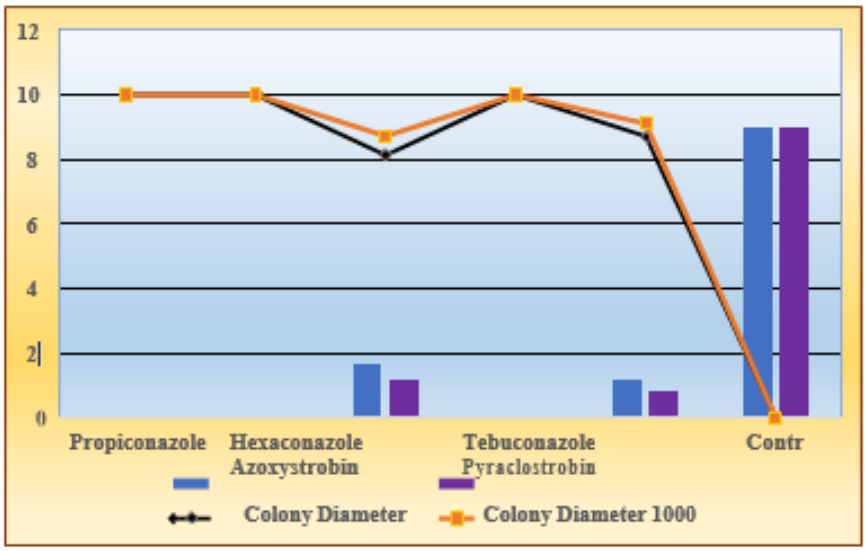

Fig 1: Efficacy of systemic fungicides against A. *macrospora* under *in vitro* conditions

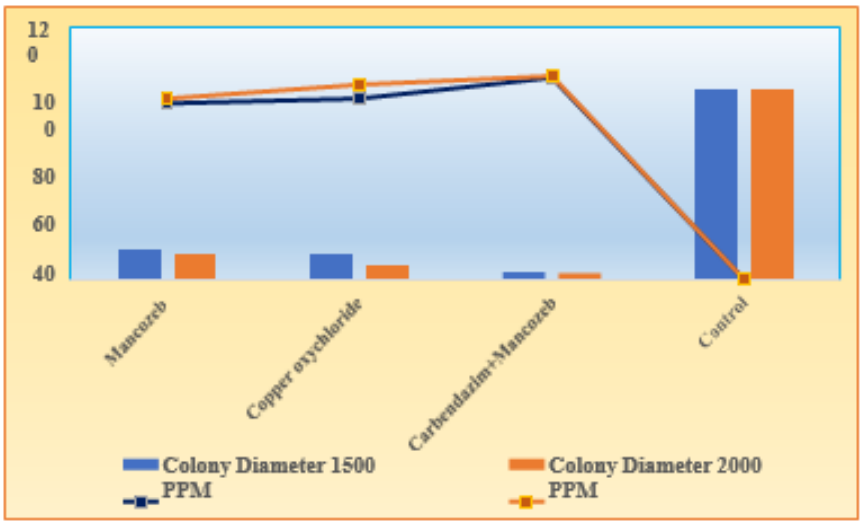

Fig 2: Efficacy of non-systemic and combined fungicides against *A. macrospora* under *in vitro* conditions

Plate 1(a): Efficacy of systemic fungicides at 500 ppm on radial mycelial growth and inhibition of *Alternaria macrospora* under *in vitro* conditions

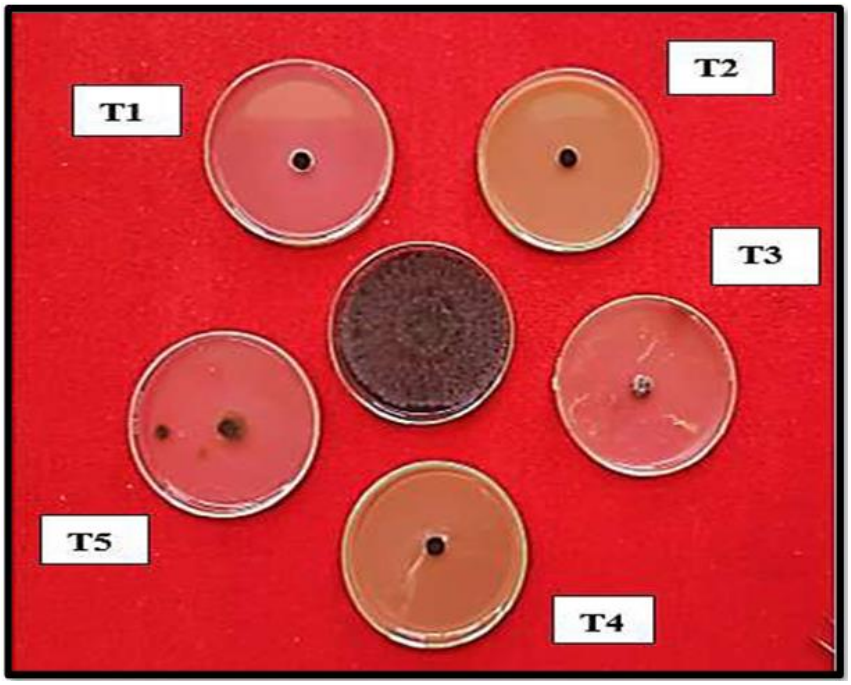

Plate 2(b): Efficacy of systemic fungicides at 1000 ppm on radial mycelial growth and inhibition of *Alternaria macrospora* under *in vitro* conditions

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