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Evaluation of fungicides, botanicals and bioagents against Alternaria leaf blight caused by *Alternaria macrospora* in cotton

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ABSTRACT : Six fungicides (@ 500, 1000, 1500 ppm), botanicals (@5, 10, 15%) and bioagents were evaluated *in vitro* against *Alternaria macrospora* causing leaf blight of cotton. All the treatments significantly inhibited mycelial growth of the test fungus over untreated control. Among the fungicides tested, Thiram recorded significantly highest mean growth inhibition (90.42%) followed by Captan (82.04%) and Mancozeb (79.88%).Of the botanicals tested, *Allium sativum* was found most inhibitory and recorded highest mean growth inhibition (37.47%) followed by *Allium capa* (34.97%) and *Oscimum sanctum* (32.86%). Among the bioagents evaluated, significantly highest mycelial growth inhibition (63.64%) was recorded with *Trichoderma viride* followed by *T. koningii* (62.33%) and *Pseudomonas fluorescens* (62.27%) both of which were *on par*.

Key words : *Alternaria macrospora,* bioagents, botanicals, fungicides, inhibiton, leaf blight/spot

Cotton (*Gossypium* spp), popularly known as 'White Gold' has been the principal commercial and industrial crop of India. In India, all four major species of cotton are grown commercially, of which major area is under *G. hirsutum* varieties and hybrids. The productivity of cotton in Maharasthra is far below than that of national productivity. Of the various factors responsible for low cotton yields in Maharasthra, diseases induced by phytopathogens (Fungi, bacteria, viruses, nematodes) are the major one which account for 20 to 30 per cent losses in cotton. Among the fungal disease of cotton, Alternaria blight caused by *Alternaria macrospora* Zimm is an emerging major foliar disease in *Bt* cotton and all four cultivated species of cotton are known to suffer from this disease (Zanjare *et al.,* 2005). The disease has been reported to cause 5-35 per cent losses in cotton yield (Zanjare *et al.,* 2005; and More *et al.,* 2010). Considering the importance of Alternaria leaf blight disease, the present investigation was carried out with an view to find out the efficiency of the fungicides biocontrol agents and botanicals/phytoextracts against *Alternaria macrospora.*

Evaluation of fungicides and botanicals : The efficacy of fungicides and botanicals was evaluated *in vitro* against *A. macrospora* applying poisoned food technique and using potato dextrose agar (PDA) as basal medium. Fungicides *viz.,* Thiram 75 WP, Captan 50 WP, Copper oxychloride 50 WP, Mancozeb 75 WP, Carbendazim 50 WP and Chlorothalonil 75 WP were evaluated each @ 500,1000 and 1500 ppm. The fungicide amended PDA medium was poured (20 ml/plate) aseptically in sterilized glass petriplates (90 mm dia) and allowed to solidify. All the test fungicides and their concentration were replicated thrice and the experiment was planned with complete randomized design (CRD). A suitable untreated control was maintained by pouring the petriplate with plain PDA without fungicide. These plates were then inoculated aseptically with 5 mm culture disc of the test fungus obtained from a week old actively growing pure culture of *A.macrospora,* by placing on solidified PDA in inverted position in the centre of petriplates and were incubated at 26+ 2^0C temp. The observations on colony dia were recorded at 24 h interval and continued till the untreated control plate was fully covered with mycelial growth of the test fungus. The per cent growth inhibition of the test fungus over untreated control was calculated.

$$\text{Per cent inhibition} = \frac{C-T}{C} \times 100$$

PI= Per cent inhibiton
C = Growth in control plate
T= Growth in treated plate

Botanicals/plant extracts *viz. neem* leaf (*Azadirachata indica*), *tulsi* leaf (*Oscimum sanctum*), ginger rhizome (*Zingiber officinale*),

Ashoka leaf (*Polyalthia longifolia*), onion bulb (*Allium cepa*) and garlic cloves (*Allium sativum*) were evaluated against *A.macrospora,* applying Poisoned food technique. Aqueous leaf extracts of the test botanicals were prepared by grinding with mixture cum grinder. The 100 g washed leaves of three (*Neem, Tulsi*, Ashoka) botanicals, ginger rhizome, garlic cloves and onion bulbs were macerated in 100ml distilled water (w/v) separately and the macerate was filtered through double layered muslin cloth. Each of the filtrate obtained was further filtered through Whatman filter paper No. 1 using funnel and volumetric flasks (100 ml cap) The final clear extracts/ filtrates obtained formed the standard plant extract of 100 per cent conc. These extracts were evaluated (@ 5, 10 and 15% each).

PDA medium amended separately with plant extract was then poured into sterile petriplates (20 ml/plate) and three replications were maintained and PDA was allowed to solidify at room temperature. The plate containing plain PDA without any plant extract was maintained as untreated control. All the treatments and control plates were aseptically inoculated by placing in the centre a 5 mm mycelial disc obtained from a week old actively growing pure culture of *A.macrospora.* All these plates were then incubated @ 26+ 2^0C till the untreated control plate was fully covered with mycelial growth of the test fungus. Observations on colony dia were recorded and per cent growth inhibition over untreated control was calculated.

Evaluation of bioagents : The antagonistic potential of 5 bioagents *viz, Trichoderma viride*, *T.harzianum*, *T.hamatum*, *T.koningii*, *T.lingorum* and *P.fluorescens* against *A.macrospora* was evaluated by Dual culture technique. Seven days old cultures of the test bioagents and *A.macrospora* multiplied on PDA were used for the study. Five mm discs of culture growth of the test fungus and bioagent were cut out with sterilized cork borer. Two culture discs, one each of test fungus and bioagents were placed aseptically at equidistance and exactly opposite with each other on solidified PDA in petriplates and all the plates were incubated at 26+2^0C. PDA plate inoculated only with the culture disc of test fungus was maintained as untreated control.

Observations on linear mycelia growth of the test fungus and bioagents were recorded at an interval of 24 h and continued till untreated control plate was fully covered with mycelial growth of the test fungus. Per cent growth inhibition of the test fungus over untreated control was calculated as below,

$$PI = \frac{\text{Colony growth in control - Colony growth in intersecting place}}{\text{Colony growth in control}} \times 100$$

PI- Per cent growth inhibition

Efficacy of fungicides : Results (Table1) revealed that all the fungicides tested were found effective and recorded significant growth inhibition of *A.macrospora* over untreated control. Further, it was found that per cent inhibition of the test fungus was increased with increase in conc of the fungicides tested.

As regards per cent growth inhibition of the test fungus, all the fungicides tested at various conc were found fungistatic/fungicidal against *A. macrospora* and significantly inhibited mycelial growth over untreated control. Further, the per cent inhibition of the test fungus recorded with fungicides tested was found to be increased with increase in their conc.

Among the fungicides tested, Thiram was found most effective which recorded maximum growth inhibition of 82.38, 94.44 and 94.44 per cent, at 500, 1000 and 1500 ppm respectively, with significantly highest mean growth inhibition (90.42%) of the test fungus. The second and third best fungicides found were Captan and Mancozeb which recorded growth inhibition of 69.55, 82.13 and 94.44; 69.41, 81.41 and 88.83 per cent, respectively at 500, 1000 and 1500 ppm.

The mean mycelial growth inhibition of the test fungus recorded with Captan and Mancozeb were 82.04 and 79.88, respectively.Rest of the fungicides tested were also found effective and recorded mean mycelial growth inhibition of 77.75 (Carbendazim), 74.52 (Chlorothalonil) and 71.75 (Copper oxychloride) per cent as against untreated control (00.00% inhibition).

Efficacy of botanicals/plant extracts : Result (Table1) revealed that all the test botanical extracts were found antifungal against

the test fungus and their inhibitory effect was found to be increased with increase in their conc.

As regards per cent growth inhibition of the test fungus, all the botanicals tested at various conc were found antifungal against *A.macrospora* and significantly inhibited mycelial growth over untreated control. Among the botanicals tested, *A. sativum* (Garlic) was found most effective which recorded maximum growth inhibition of 26.88, 39.33 and 46.21 per cent, respectively at 5, 10 and 15 per cent concentration, with significantly highest mean growth inhibition (37.47%) of the test fungus.The second and third best botanicals found were *A. cepa* (Onion) and *O. sanctum* (*Tulsi*) which recorded growth inhibition of 24.74, 36.30 and 43.87; 21.94, 31.48 and 38.18 per cent, respectively at 5, 10 and 15 per cent conc. The mean growth inhibition of the test fungus recorded with all the botanicals tested was ranged from 23.60 (*P.longifolia*) to 37.47 per cent (*A.sativum*). However, the significantly highest mean growth inhibition (37.47%) was recorded with *A.sativum* (Garlic) followed by *A. cepa* (34.97%), *O.sanctum* (32.86%), *Z.officinale* (28.74) and *A.indica* (25.28%).

Efficacy of bioagents : Results (Table1) revealed that all the five species of fungal antagonist *Trichoderma* and one bacterial antagonist *P. fluorescens* evaluated were found fungistatic against. *A. macrospora* and exhibited significant growth inhibition of the test fungus over untreated control. However, significantly highest mycelial growth inhibition (63.64%) was recorded with *T.viride*. The second and third best bioagents found were *T.koningii* and *P.fluorescens* which recorded 62.33 and 62.27 per cent growth inhibition, respectively of the test fungus and both of which were *on par*. This was followed by *T.harzianum* (60.88%) and *T.hamatum* (59.11 % inhibition) both of which were *on par*. However, *T. lignorum* was found comparatively less effective which recorded 36.58 per cent inhibition of the test fungus.Thus, all the fungicides, botanicals and bioagents tested were found fungistatic/ antifungal against *A.macrospora.* However, among the fungicides Thiram and Captan, among the botanical *A.sativum* (Garlic) and *A.cepa* (Onion) and among the bioagents *T.viridae*, *T.koningii* and *P.fluorescens* were found most effective against the test fungus.

Result obtained in present study on antifungal effects of the test fungicides against *A.macrospora* are in consonance with those reported earlier by many workers. Fungicide Thiram was reported effective against *A.alternata*, *A.carthami* and *A.macrospora* earlier by Gangurde *et al.,* (2003). Fungicides, Mancozeb, Captan, and Carbendazim were reported inhibitory against *A.macrospora*, *A.carthami* and *A.alternata* earlier by Gangurde *et al.,* (2003), and Ramegowda *et al.,* (2007).

The antifungal effects of the botanicals tested against the test fungus may be attributed to the presence of antifungal compounds like phenols, tannins, alkaloids and non volatile substances. Antifungal effects of the botanical *viz, A.sativum*, *A.cepa*, *O.sanctum* and *A.indica* found in present study against *A.macrospora* are in comformity with those reported earlier by several workers. *A.sativum* (Garlic) clove extracts was reported antifungal against *A.tenuissina A.alternata A.chlymadospora*. The leaf extracts of *A.indica* (*Neem*) and *O.sanctum* (*Tulsi*) were reported fungistatic against *A.macrospora, A.brassicola, A.alternata* and *Alternaria spp*. earlier by (Babu *et al.,* 2001).

The antagonistic effect of the fungal biocontrol agents *Trichoderma* spp and bacterial biocontrol agent *P.fluorescens* against *A.macrospora* found in present study may be attributed to the mechanisms *viz.,* competition, lysis, mycoparasitism, antibiosis and production of volatile and non volatile compounds by the bioagents. Results of the present study obtained on antifungal effects of the test bioagents against *A.macrospora* are in agreement with those reported earlier by several workers. Chidambaram *et al.,* (2002), Gangurde *et al., (2003)*, Ramegowda *et al.,* (2007) and Dalpati *et al.,* (2010) recorded significant inhibition of *A.macrospora* and other *Alternaria* sp by *T.viride*, *T.harzianum*, *T.koningii* and *P.fluoerescens*.

Thus, from the perusal of the results obtained on efficacy of the fungicides, botanicals and bioagents against *A.macrospora* causing leaf blight in cotton, it can be concluded that fungicides Thiram, Captan and Manocozeb; botanicals *A.sativum*, *A.cepa* and *O.sanctum* and bioagents *T.viride, T.koningii*, *T.harzianum* and *P.fluorescens* were found effective against

Table 1. Efficacy of fungicides, botanicals and bioagents against Alternaria macrospora Zimm.

Fungicides	Per cent inhibition (ppm)			**Mean inhibition (%)**	Botanicals/ Plant extracts	Per cent inhibition (Conc.%)			**Mean inhibition (%)**	Bioagents	Per cent inhibition
	500	1000	1500			5	10	15			
Thiram	82.38	94.44	94.44	**90.42**	*Neem*	15.71	27.54	32.61	**25.28**	*T. viride*	63.64
	-55.47	-70.79	-70.79		(*A.indica*)	-9.02	-15.97	-19.03			-39.51
Captan	69.55	82.13	94.44	**82.04**	Onion	24.74	36.3	43.87	**34.97**	*T. harzianum*	60.88
	-44.06	-55.21	-70.79		(*A.cepa*)	-14.32	-21.27	-26.01			-37.5
Copper oxychloride	60.54	71.76	82.95	**71.75**	Garlic	26.88	39.33	46.21	**37.47**	*T. hamatum*	59.11
	-37.24	-45.84	-56.05		(*A.sativum*)	-15.59	-23.34	-27.51			-36.16
Mancozeb	69.41	81.41	88.83	**79.88**	*Tulsi*	21.94	31.48	38.18	**32.86**	*T. koningii*	62.33
	-43.94	-54.5	-62.64		(*O. sanctum*)	-12.67	-18.34	-22.44			-38.55
Carbendazim	68.88	76.97	86.65	**77.5**	Ginger	19.96	29.77	36.5	**28.74**	*T. lignorum*	36.58
	-43.53	-50.32	-60.04		(*Z. officunale*)	-11.51	-17.32	-21.4			-21.61
Chlorothalonil	64.52	76.04	83	**74.52**	Ashoka	14.66	23.91	32.24	**23.6**	*P. fluorescens*	62.27
	-40.17	-49.49	-56.1		(*P. longifolia*)	-8.42	-13.81	-18.8			-38.51
Control	0	0	0	**0**	Control	0	0	0	**0**	Control	0
(untreated)	0	0	0			0	0	0			0
SE +	0.27	0.45	0.38			0.33	0.34	0.2			0.2
P=0.05	0.82	1.36	1.16			1.01	1.04	0.62			0.61

A.macrospora under *in vitro* evaluation needs to be confirmed further after which could be integrated for effective and economical management of Alternaria blight (*A.macrospora*) of cotton under field conditions.

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