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In-vitro evaluation of fungicides, bioagents and aqueous leaf extracts against *Alternaria* leaf blight of cotton

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ABSTRACT

All the six fungicides *viz.,*Mancozeb (75% WP); Carbendazim (50WP), Copper oxychloride (50WP), Captan (50 WP), Thiram (75 % WP), Chlorothalonil (75 WP) evaluated *in vitro* (@500, 1000 and 1500 ppm) were found effective against *A. macrospora* and caused significant inhibition of test pathogen over untreated control. However, Thiram was found most effective and recorded significantly highest mean mycelial inhibition (90.42%). This was followed by the fungicides, Captan (82.04%), Mancozeb (79.88%), Carbendazim(77.5%), Chlorothalonil (74.52%) and copper oxychloride (71.75%). All the five fungal and one bacterial bioagents/ antagonists evaluated *in vitro* against *A. macrospora* were found antifungal/ antagonistic against the test pathogen. However, *T. viride* was found most effective and recorded least linear mycelial growth (32.72 mm) with corresponding significantly highest mycelial inhibition (63.64%) of the test pathogen. The second and third best bioagents found were *T. koningii* and *P. fluorescens,* which recorded linear mycelial growth, respectively of 33.90 mm and 33.95 mm with corresponding mycelial inhibition, respectively of 62.33 and 62.27 per cent. All the six botanicals/plant extracts evaluated *in vitro* (@5, 10 and 15 % each) were found fungistatic/ fungicidal against. *A.macrospora.* However, significantly least mean radial mycelial growth (56.18mm) and significantly highest mean mycelial inhibition (37.47%) was recorded with Garlic. The second and third best botanicals found were onion and Tulsi which recorded second and third least mean radial mycelial growth, respectively of 58.52 mm and 62.51 mm with

corresponding mean mycelial inhibition of 34.97 and 32.86 percent, respectively.

1. Introduction

Cotton (*Gossypium* spp) is popularly known as "white gold" and is premier cash crop of most of the SAARC countries with an enormous potential of employment generation, both in rural and urban sectors. India occupies first place in area and second place in cotton production after USA in the world. During 2009-2010, cotton cultivated area was 103.10 lakh hectare with production of 295 lakh bales and productivity 486 kg lint /ha. In India, Maharashtra ranks first in area and second in production. During 2009-2010, Maharashtra's total cultivated area was 35.03 lakh hectares with production of 63 lakh bales and productivity was 306 kg lint/ha (Anonymous, 2010).

Among the several factors responsible for reduction in yield and quality deterioration of cotton in India, diseases occupy a vital place. Amongst the various fungal, bacterial, viral and deficiency diseases, *Alternaria* leaf blight, and other leaf spotting fungi poses an alarming situation, but very scanty work has been done on this disease. In India, leaf spot of cotton (*Alternaria macrospora),* Zimm was reported firstly by Uppal *et al.* (1935), from Bombay, later Rane and Patel (1956), reported it from Poona and Ahmednagar. Bashi *et al*.(1983), reported that epidemics of *Alternaria* leaf spot in Israel could decrease the yield of Pima-S-5 by 25 per cent. Vasudeva (1960), reported that disease was serious on three cotton varieties of *G. hirsutum*, the other cultivated species of *Gossypium* being resistant. *Alternaria* blight (*A.macrospora*) has been reported about 20-30 per cent losses in seed cotton yield.(Srinivas, 1994; Chauhan *et al*, 1997; Mayee and Mukewar, 2007). Considering occurrence and losses caused by *Alternaria macrospora* in cotton, present investigations were planned and undertaken.

2. Materials and methods

The efficacy of fungicides earlier reported effective against *A. macrospora* were evaluated *in vitro* by applying Poisoned food technique (Nene and Thapliyal, 1993) and using PDA as basal medium. PDA medium was used as a basal medium for the fungicidal study. It was prepared in 250 ml conical flask. 100ml medium was taken in each flask. The medium then sterilized at 15 lbs vapour pressure for 15 minutes. Required quantity of test fungicides were calculated and added in the sterilized medium separately. Flasks containing Poisoned medium were shaken well to have even and uniform distribution of the fungicides. About 20 ml of Poisoned PDA was poured in each of the sterilized petri plates and allowed to solidify. The plates were inoculated by pure culture of *Alternaria macrospora*. For this purpose, 5 mm disc of one week old culture was cut with a sterilized cork borer. The disc was lifted and transferred aseptically in the centre of Petriplates containing the medium with test fungicides. Three plates were maintained for each treatement. The control plates without fungicides were also inoculated and incubated. Treated plates were incubated at 26 ± 2^0C temperature. The observations on colony diameter were recorded after 8 days.

The antagonistic potential of bioagents *viz*, *Trichoderma viride, T. harzianum, T. lignorum, T. koningii, T.hamatum,* and *Pseudomonas fluorescence* against *A. macrospora* were tested by dual culture technique on PDA medium as per procedure described by Dalpati *et al.* (2010). For this 20 ml of sterilized, melted and cooled medium was poured in each petriplate (90 mm). Allowed to solidify and the plates were inoculated with 5 mm disc of 7 days old growth of fungal biocontrol agents with the help of sterilized cork borer and subsequently inoculated with 5 mm disc of 7 days old culture of *Alternaria macrospora* at opposite corner of the plates, keeping 15 mm distance from peripheri. For bacterial antagonist *Pseudomonas fluorescence* was streaked with the help of sterilized inoculating needle at one end of the PDA petriplate. After 24 hrs of incubation just opposite to the bacterial streak a 5 mm disc of pathogen was placed with the help of sterilized cork borer. The inoculations of pathogen alone in the centre in the plates serve as control. Three replications of each treatment including the control were maintained. These plates were incubated at 26 ± 2^0 C in incubator.

Six botanicals were evaluated at 5, 10 and 15% concentrations for their inhibitory potential on mycelial growth of *Alternaria macrospora* under *in vitro* condition by Poison food technique (Shravelle, 1961). Three replications were maintained for each treatment. 05 ml, 10 ml and 15 ml of test phytoextracts were poured into

100 ml molten PDA in a conical flask separately. The prepared solution was poured into petriplates and allowed to cool. 5 mm disc of *A. macrospora* was placed at the centre under aseptic condition. Suitable control plates of each treatment were maintained by growing the culture on PDA without the botanicals. These petriplate were incubated at 26±2^{0}C till the growth of culture in controls covered entire petriplate.

The inhibition zone was calculated by using the following formula and the data was statistically analyzed.

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

Where,
PI= Per cent inhibition
C= Growth in control plates
T= Growth in plates treated with fungicides.

3. Results and discussion

A total of six fungicides *viz*., Thiram 75 WP, Captan 50 WP, Copper oxychloride 50 WP, Mancozeb 75 WP, Carbendazim 50 WP, and Chlorothalonil 75 WP were evaluated (@ 500, 1000 and 1500 ppm each), *in vitro* against *A. macrospora*, applying Poisoned food technique (Nene and Thapliyal, 1993) and using Potato dextrose agar as basal medium. All the treatments were replicated thrice and a suitable untreated control was maintained.

3.1. Radial mycelial growth

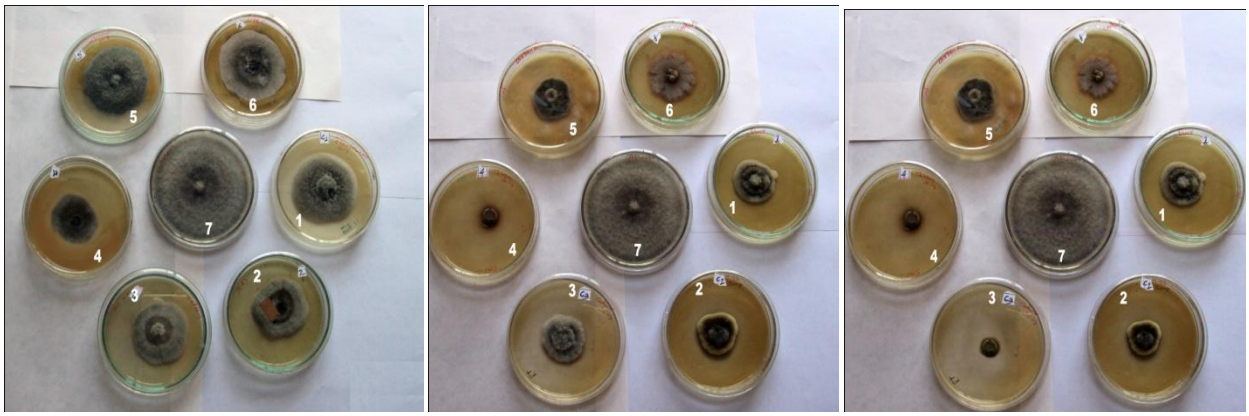
Results (Table 1) revealed that all the fungicides tested recorded a wide range of radial mycelial growth of the test pathogen (PLATE I) and it was varied with their concentrations used. At 500 ppm, radial mycelial growth of the test pathogen was ranged from 15.85 mm (Thiram) to 35.51 mm (Copper oxychloride). However, it was maximum with Copper oxychloride (35.51 mm), and this followed by Chlorothalonil (31.93 mm) and Carbendazim (28 mm). Minimum radial mycelial growth was recorded with Mancozeb (27.53 mm), Captan (27.40 mm) and Thiram (15.85 mm).

Table 1
Efficacy of fungicides against *Alternaria macrospora Z*imm.

Fungicides	Colony Dia. (mm)*			Mean	Percent Inhibition			Mean
	500	1000	1500	(mm)	500	1000	1500	%
Thiram	15.85	5.00	5.00	08.61	82.38 (55.47)	94.44 (70.79)	94.44 (70.79)	90.42
Captan	27.40	16.08	5.00	16.16	69.55 (44.06)	82.13 (55.21)	94.44 (70.79)	82.04
Copper oxychloride	35.51	25.41	15.34	25.42	60.54 (37.24)	71.76 (45.84)	82.95 (56.05)	71.75
Mancozeb	27.53	16.73	10.05	18.10	69.41 (43.94)	81.41 (54.50)	88.83 (62.64)	79.88
Carbendazim	28.00	20.72	12.01	20.24	68.88 (43.53)	76.97 (50.32)	86.65 (60.04)	77.5
Chlorothalonil	31.93	21.56	15.30	22.93	64.52 (40.17)	76.04 (49.49)	83 (56.10)	74.52
Control	90.00	90.00	90.00	90.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00
SE ±	0.29	0.42	0.33		0.27	0.45	0.38	
CD	0.88	1.29	1.01		0.82	1.36	1.16	

*Average of three replications
Figures in parenthesis are arc sine values.

At 1000 ppm, radial mycelial growth of the test pathogen was ranged from 05.00 mm (Thiram) to 25.41 mm (Copper oxychloride). All the fungicides tested exhibited similar trend of radial mycelial growth as that of 500 ppm. However maximum radial mycelial growth was recorded with Copper oxychloride (25.41 mm), and was followed by Chlorothalonil (21.56 mm) and Carbendazim (20.72 mm). Least radial mycelial growth of 16.73 mm, 16.08mm and 5.00 mm was recorded with Mancozeb, Captan and Thiram, respectively. At 1500 ppm, radial mycelial growth was ranged from 5.00 mm (Thiram) to 15.34 mm (Copper oxy chloride). All the fungicides tested (@ 1500 ppm) significantly recorded reduced radial mycelial growth as compared to 500 and 1000 ppm. The mean radial mycelial growth recorded with the fungicides tested (@ 500, 1000, 1500 ppm) was ranged from 08.61 mm (Thiram) to 25.42 mm (Copper oxychloride). However, highest mean radial mycelial growth was recorded with Copper oxychloride (25.42 mm) which was followed by Chlorothalonil (22.93 mm) and Carbendazim (20.24 mm). The least mean radial mycelial growth was recorded with Mancozeb (18.10 mm), which was followed by Captan (16.16 mm) and Thiram (8.61 mm).



A) 500 ppm B) 1000 ppm C) 1500 ppm

Plate I. *In vitro* effect of fungicides at 500 ppm(A),1000 ppm(B)and 1500 ppm(C) on mycelial growth and inhibition of *Alternaria macrospora* Zimm. 1) Chlorothalonil, 2) Mancozeb, 3) Captan, 4) Thiram, 5) Carbendazim, 6) Copper oxychloride, 7) Control.

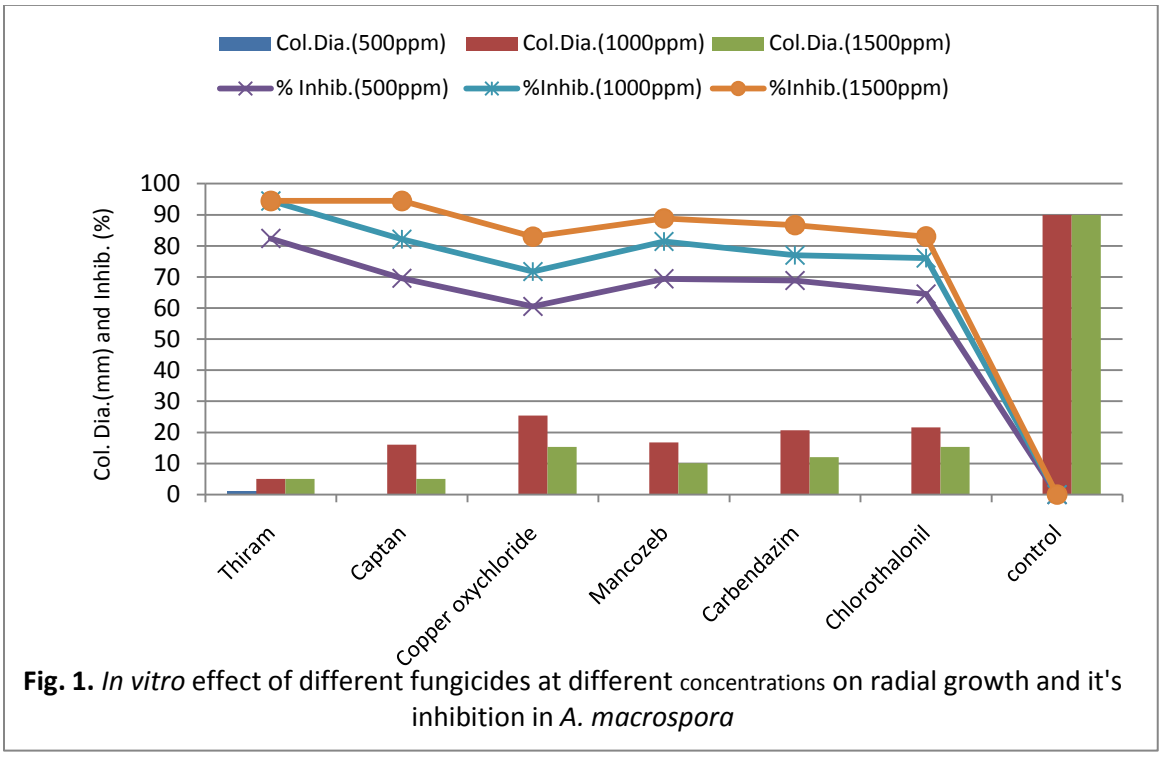


Fig. 1. *In vitro* effect of different fungicides at different concentrations on radial growth and it's inhibition in *A. macrospora*

3.2. Mycelial inhibition

Results (Table 1) revealed that all the fungicides tested (@ 500, 1000 and 1500 ppm) significantly inhibited mycelial growth of the test fungus over untreated control. Further, it was found that per cent mycelial inhibition of the test pathogen was increased with the increase in concentrations of the fungicides tested (PLATE I and Fig.1).

At 500 ppm, per cent mycelial growth inhibition was ranged from 60.54 (Copper oxychloride) to 82.38 (Thiram). However, highest percentage mycelial inhibition was recorded with Thiram (82.38%). This was followed by the fungicides, Captan (69.55%), Mancozeb (69.41%), Carbendazim (68.88%), Chlorothalonil (64.52%) and Copper oxychloride (60.54%). At 1000 ppm, similar trend of mycelial growth inhibition with the test fungicides was recorded and it was ranged from 71.76 percent (Copper oxychloride) to 94.44 percent (Thiram). However, highest percentage mycelial inhibition was recorded with Thiram (94.44%). This was followed by Captan (82.13 %), Mancozeb (81.41), Carbendazim (76.97%), Chlorothalonil (76.04%) and Copper oxychloride (71.76%). At 1500 ppm, the percentage of mycelial inhibition was ranged from 82.95 percent (Copper oxychloride) to 94.44 percent (Thiram). Mean percentage mycelial inhibition recorded with all the fungicides tested (@500, 1000 and 1500 ppm) was ranged from 71.75 (Copper oxychloride) to 90.42 (Thiram). However, Thiram was found to be most fungistatic which recorded significantly highest mean mycelial inhibition of 90.42 percent. This was followed by Captan (82.04%), Mancozeb (79.88%), Carbendazim (77.5%), Chlorothalonil (74.52 %) and Copper oxychloride (71.75%). Thus, all the fungicides tested were found fungistatic against *Alternaria macrospora* and significantly inhibited mycelial growth of the test pathogen over untreated control. However, Thiram recorded highest meanmycelial inhibition (90.42%) followed by Captan (82.04%) and Mancozeb (79.88%). Similar *in vitro* fungistatic effects of the test fungicides against *A.macrospora* infecting cotton and other *Alternaria* spp. infecting many other crops were reported earlier by several workers (Gupta and Prasad, 1968; Bhaskaran and Shanmugam, 1973; Padmanabhan and Narayanswamy, 1976; Siganmathi and Ekbote, 1960; Padule and Shinde, 1989; Gangurde, 2003; Ramegowada *et al.,* 2007).

3.3. Efficacy of bioagents

Results (Table 2 and Fig.2) revealed that all the bioagents evaluated exhibited fungistatic/antifungal activity against. *A. macrospora* and significantly inhibited its mycelial growth over untreated control (PLATE-II). Of the antagonists tested, *T.viride* was found most effective and recorded least linear mycelial growth (32.72 mm) with highest mycelial inhibition (63.64%) of the test pathogen over untreated control (90.00 mm and 00.00%). The second and third best antagonists found were *T. koningii* and *P.fluorescens* which recorded mycelial growth of 33.90 mm and 33.95 mm respectively and inhibition of 62.33 and 62.27 percent, respectively both of which are at par. This was followed by *T. harzianum and T. hamatum* with colony growth respectively of 35.20 and 36.80 mm, and correspond growth inhibition of 60.88 and 59.11 per cent, respectively. *T. lignorum* was found comparatively less effective with 56.83 mm colony diameter and 36.58 per cent inhibition of the test pathogen.Thus, all the fungal and bacterial antagonists/bioagents evaluated *in vitro* were found fungistatic /antifungal against *A. macrospora* and caused significant reduction in the linear mycelial growth of the test pathogen over untreated control.

Results of the present study on inhibitory effects of the test antagonists: *Trichoderma* spp. and *P. fluorescens* are in conformity with those reported earlier by several workers (Lokesh and Hiremath, 1988; Palazon and Palazon, 1988; Chidambaram *et al,* 2002; Gangurde *et al., 2003;* Mehtre *et al,* 2003; Woo *et al,* 2005; Ramegowda *et al,* 2007; Bhattiprolu and Prasada Rao, 2009; Dalpati, 2010).

3.4. Efficacy of plant extracts

A total of six botanicals/plant extracts *viz*., Neem (*Azadirachta indica*), Onion (*Allium cepa*), Garlic (*Allium sativum*), Tulsi (*Oscimum sanctum*), Ginger (*Zingiber officinale*), and Ashoka (*Polyalthia longifolia*) were evaluated (@ 5, 10 and 15%) *in vitro* against *A. macrospora* applying Poisoned food technique and using PDA as a basal medium. All the treatments were replicated thrice and a suitable untreated control was maintained.

3.5. Radial mycelial growth

Results (Table 3 and Fig 3) revealed that all the botanicals/plant extracts tested exhibited the varied range of radial mycelial growth of the test pathogen (PLATE-III) depending upon their concentrations used. At 5 per cent, radial mycelial growth of the test pathogen was ranged from 65.80 mm (Garlic) to 76.80 mm (Ashoka). However, it was maximum with Ashoka (76.80 mm). This was followed by Neem (75.86 mm), Ginger (72.03 mm), Tulsi (70.25

mm), Garlic (65.80 mm) and Onion (67.73 mm). At 10 percent, radial mycelial growth of the test pathogen was ranged from 54.33 mm (Garlic) to 68.48 mm (Ashoka). All the plant extracts tested (@ 10 percent), exhibited similar trend of radial mycelial growth as that of 5 percent. However, maximum radial mycelial growth was recorded with Ashoka (68.48 mm) and was followed by Neem (65.21 mm) Ginger (63.20 mm), Tulsi (61.66 mm), Garlic (54.33 ml), and Onion (57.33 mm). At 15 percent, radial mycelial growth was ranged from 48.41 mm (Garlic) to 60.98 mm (Ashoka). All the botanicals tested (@ 15%), recorded reduced mycelial growth as compared to 5 and 10 percent. The mean radial mycelial growth recorded with all the plant extracts tested (@ 5, 10 and 15%) was ranged from 56.18 mm (Garlic) to 68.75 mm (Ashoka). However, highest mean radial mycelial growth was recorded with Ashoka (68.75 mm), and was followed by Neem (67.24 mm), Ginger (64.12 mm), Tulsi (62.51 mm), Garlic (56.18 mm) and Onion (58.52 mm).

Table 2
Efficacy of bioagents against *Alternaria macrospora* Zimm.

Bioagents	Colony Dia. (mm)*	% Inhibition
T. viride	32.72	63.64 (39.51)
T. harzianum	35.20	60.88 (37.50)
T. hamatum	36.80	59.11 (36.16)
T. koningii	33.90	62.33 (38.55)
T. lignorum	56.83	36.58 (21.61)
P. fluorescens	33.95	62.27 (38.51)
Control	90.00	00.00 (00.00)
SE ±	0.25	0.20
CD	1.65	0.61

*Average of three replications
Figures in parenthesis are arc sine values.

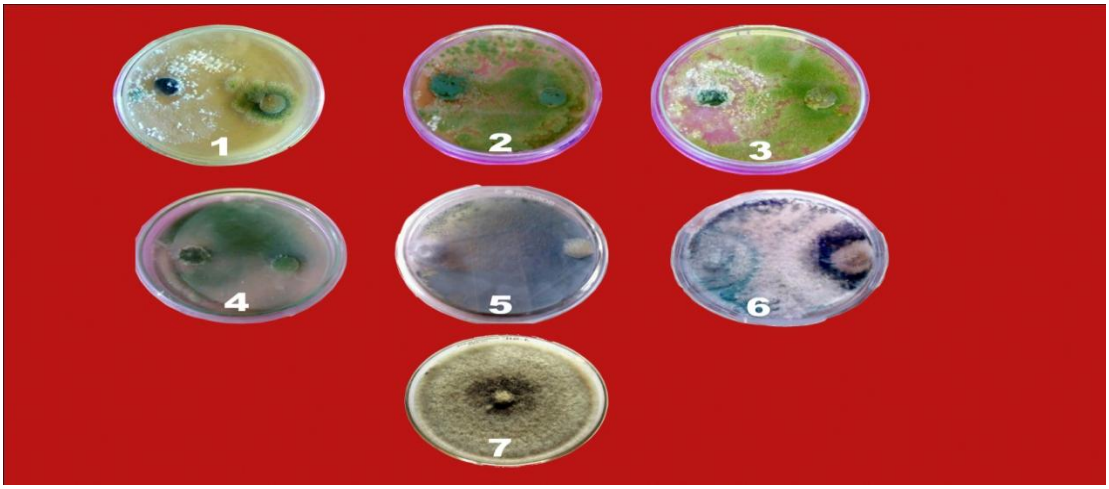


Plate II: *In vitro* effect of bioagents on mycelial growth and inhibition of *Alternaria macrospora* Zimm. 1) *T. hamatum*, 2) *T.koningii*, 3) *T. lignorum*, 4) *P. fluorescens*, 5) *T.viride*, 6) *T.harzianum*, 7) control

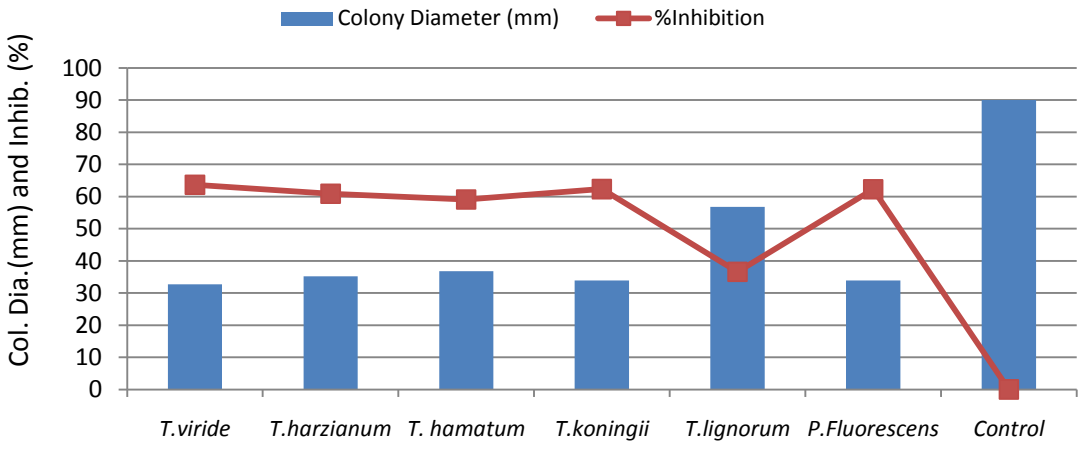


Fig. 2. *In vitro* effect of different bioagent s on radial growth and it's inhibition in *A. macrospora.*

3.6. Mycelial inhibition

Result (Table 3 and Fig 3) revealed that all the plant extracts tested (@ 5, 10 and 15 percent), significantly inhibited mycelial growth of the test fungus over untreated control and percent mycelial inhibition was increased with increase in concentrations of the botanicals tested (PLATE-VIII). At 5 percent, mycelial growth inhibition was ranged from 14.66 (Ashoka) to 26.88 percent (Garlic). However, significantly highest percentage of mycelial inhibition was recorded with Garlic (26.88%), and was followed by onion (24.74%). Tulsi (21.94%), Ginger (19.96%) Neem (15.71%) and Ashoka (14.66%). At 10 percent, similar trend of mycelial inhibition as that of 5 percent was recorded and it was ranged from 23.91 percent (Ashoka) to 39.33 percent (Garlic). However, significantly highest percentage of mycelial inhibition was recorded with Garlic (39.33%), and was followed by Onion (36.30%), Tulsi (31.48%), Ginger (29.77%) Neem (27.54%) and Ashoka (23.91%). At 15 percent, the percentage mycelial inhibition was ranged from 32.24 percent (Ashoka) to 46.21 percent (Garlic). Mean percentage mycelial inhibition recorded with all the test botanicals was ranged from 23.60 (Ashoka) to 37.47 percent (Garlic). However, Garlic was found to be most fungistatic which recorded significantly highest mean mycelial inhibition (37.47%), and this was followed by Onion (34.97%), Tulsi (32.86%), Ginger (28.74%), Neem (25.28%), and Ashoka (23.60%). Thus, all the plant extracts tested were found fungistatic/antifungal against *A.mcrospora and* significantly inhibited mycelial growth of the test pathogen over untreated control. However,Garlic recorded highest mean mycelial inhibition (37.47%) and was followed by onion (34.97%) and Tulsi (32.86). Results of the present study are in conformity with those reported earlier by several workers (Barnwal *et al,* 1998; Karade and Sawant, 1999; Ramegowda *et al,* 2007; Naik *et al.* 2010; Dalpati *et al.,* 2010).

4. Conclusion

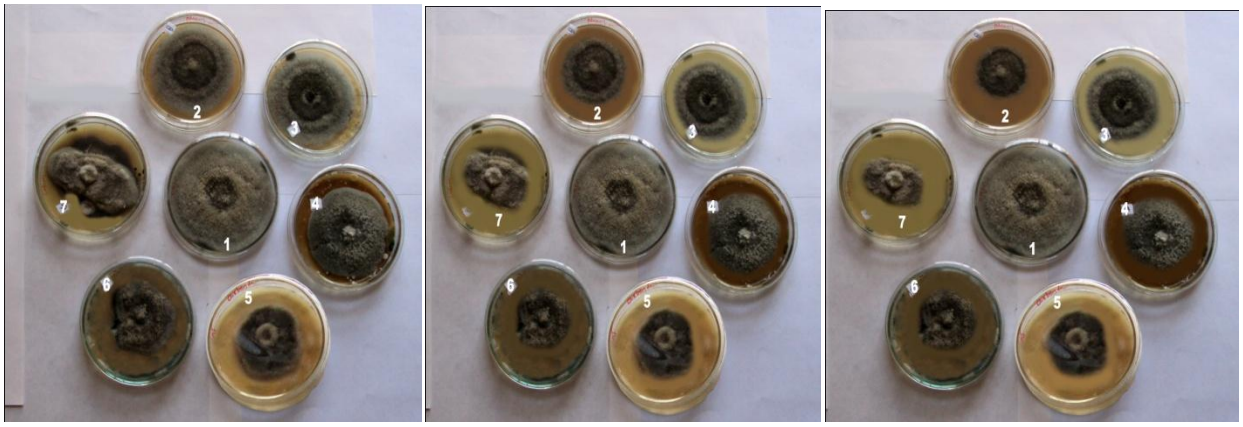
In vitro evaluation of fungicides revealed that thiram, captan and mancozeb were most effective in arresting / checking growth of *A.macrospora.* Antagonists/ biocontrol agents *viz., Trichoderma* spp. and *P. fluorescens* found fungistatic/ fungitoxic against *A. macrospora* in present studies could be exploited under field conditions for biological control of *Alternaria* blight disease of cotton. The inhibiton effects of *Trichoderma* spp. and *P.fluorescens* against *A. macrospora* may be attributed to the mechanisms *viz.* antibiosis, lysis, mycoparasitism, competition and production of volatile substances. Aqueous leaf extracts of the botanicals *viz.,* Garlic, Onion, Tulsi, Ginger, Neem and Ashoka found fungistatic/ fungitoxic against *A. macrospora* in present studies could further be popularized

commercially for economical as well as eco-friendly management/ control of *Alternaria* blight of cotton. The effectiveness of onion bulb extract may be due to presence of antifungal compounds such as cycloallin and carbohydrate propenyl sulphuric acid.

Table 3
Efficacy of plant extracts against *Alternaria macrospora* Zimm.

Plant Extracts	Colony Diameter (mm)*			Mean (mm)	Percent Inhibition			Mean %
	5%	10%	15%		5%	10%	15%	
Neem	75.86	65.21	60.65	67.24	15.71 (9.02)	27.54 (15.97)	32.61 (19.03)	25.28
Onion	67.73	57.33	50.51	58.52	24.74 (14.32)	36.30 (21.27)	43.87 (26.01)	34.97
Garlic	65.80	54.33	48.41	56.18	26.88 (15.59)	39.33 (23.34)	46.21 (27.51)	37.47
Tulsi	70.25	61.66	55.63	62.51	21.94 (12.67)	31.48 (18.34)	38.18 (22.44)	32.86
Ginger	72.03	63.20	57.15	64.12	19.96 (11.51)	29.77 (17.32)	36.50 (21.40)	28.74
Ashoka	76.80	68.48	60.98	68.75	14.66 (8.42)	23.91 (13.81)	32.24 (18.80)	23.60
Control	90.00	90.00	90.00	90.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00
SE ±	0.50	0.52	0.29		0.33	0.34	0.20	
CD	1.54	1.58	0.89		1.01	1.04	0.62	

* Average of three replications
Figures in parenthesis are arc sine values.



A) 5 % B) 10 % C) 15 %

Plate III: *In vitro* effect of plant extracts at 5 % (A), 10% (B) and 15 % (C) on mycelial growth and inhibition of *Alternaria macrospora* Zimm. 1) Control, 2) Neem 3) Ashoka 4) Tulsi 5) Ginger 6) Onion 7) Garlic.

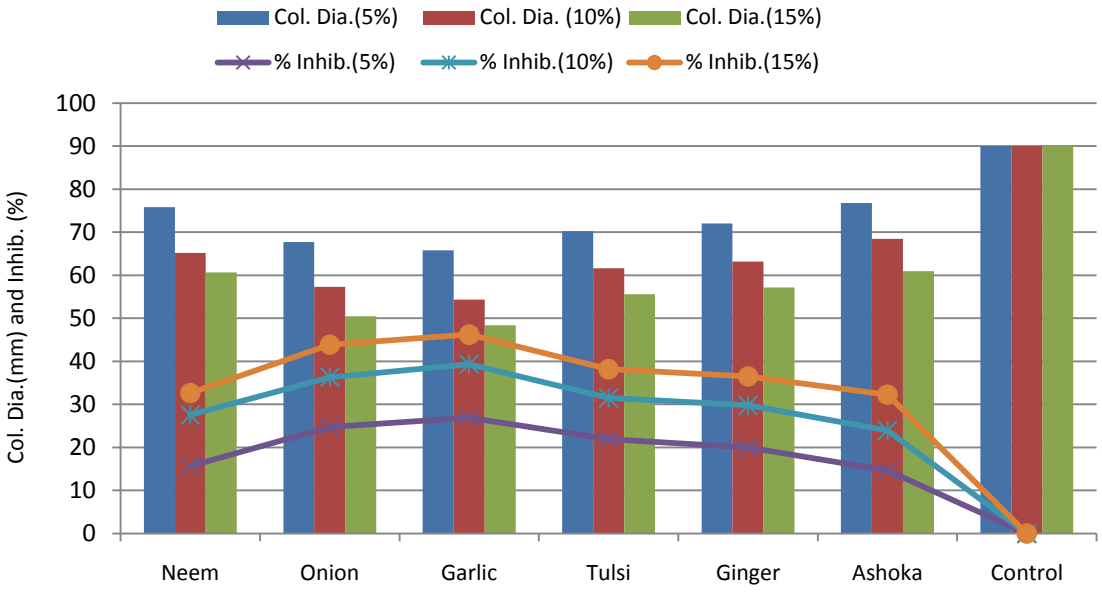


Fig. 3. *In vitro* effect of different plant extract at different concentrations on radial mycelial growth and it's inhibition in *A. macrospora*

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