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Bio-efficacy of fungicides, bioagents and plant extracts/ botanicals against *Alternaria carthami,* the causal agent of *Alternaria* blight of Safflower (*Carthamus tinctorius* L.)

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A total of eleven fungicides namely: mancozeb 75 WP, chlorothalonil 75 WP, copper oxychloride 50 WP, thiram 75 WP, captan 50 WP, difenconazole 25 EC, carbendazim 50 WP, hexaconazole 5 EC, propiconazole 25 EC, penconazole 10 EC and metalyxil + mancozeb (Ridomil) 68 WP were evaluated (at 500, 1000 and 1500 ppm each) *in vitro* against *Alternaria carthami*. All the fungicides tested caused significant inhibition at all three concentrations tested over untreated control. The average inhibition recorded with the test fungicides was ranged from 28.39% (chlorothalonil) to 94.44% (carbendazim). However, carbendazim recorded significantly highest average mycelial growth inhibition (94.44%). The second and third best fungicides found were mancozeb (85.43%) and thiram (83.33%). These were followed by hexaconazole (82.95%), ridomil (82.83%) and difenconazole (77.16%). All the six fungal and one bacterial bioagents/antagonists evaluated *in vitro* were found antifungal/antagonistic against *A. carthami*. However, *T. viride* was found most effective and recorded significantly highest mycelial growth inhibition (94.07%) of the test pathogen over untreated control. The second and third best bioagents/antagonists found were *T. hamatum* and *T. koningii* which recorded mycelial growth inhibition 85.18 and 81.11%, respectively. All the eleven plant extract/botanicals were evaluated *in vitro* (each at 10, 15 and 20%) against *A. carthami*. The mean percentage mycelial growth inhibition recorded with all the test botanicals was ranged from 19.26 (*A. racemosus*) to 62.47 (*A. sativum*) per cent. However, *A. sativum* was found most fungistatic which recorded significantly highest mean mycelial growth inhibition (62.47%). The second and third best botanicals found were *D. metal* (49.87%) and *C. longa* (46.91%). Thus, all the botanicals tested were found fungistatic antifungal and caused significant inhibition of *A. carthami* over untreated control.

Key words: *Alternaria carthami, in vitro,* fungicides, botanicals, bioagents, inhibition.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of the important oilseed crops of the world valued for its highly nutritious edible oil. Safflower seed contains about 25-32% oil. It belongs to family compositae and believes to be native of Afganistan. Safflower is known to suffer from many fungal diseases at different stage of crop growth (Bhale et al., 1998). Leaf spot/blight (*Alternaria carthami*), wilt (*Fusarium oxysporum* f. sp. *carthami*), root rot

(*Rhizoctonia batatiocla*), powdery mildew (*Erysiphe cichoracearum* DC), anthracnose (*Colletotrichum capsici*).

Out of several diseases reported on safflower, *Alternaria* blight caused by *A. carthami* is one of the most important diseases. This disease was first reported by Chowdhury (1944) at Pune in India. In India, disease plays an important role in safflower cultivation and responsible to cause 25 to 60% yield losses every year. In general, the season was suitable for the development of foliar diseases particularly for *Alternaria* leaf spot/ blight development as there were rains after sowing in Maharashtra and a disease severity from 10 to 95% was observed. In Marathwada region of Maharashtra, *Alternaria* leaf spot intensity of 30 to 40%, while in Karnataka and Andhra Pradesh maximum intensity of *Alternaria* leaf spot upto 25 and 20%, respectively

The disease has been reported to cause seed yield losses to the tune of 10-25% (Indi et al., 1988). Under severe conditions, it has been reported to cause 50% loss in yield (Indi et al., 1986). An extensive survey work carried out by Deokar et al. (1991) revealed the predominance of *Alternaria* leaf spot disease on safflower in the traditional safflower growing areas in the scarcity zone of Maharashtra.

The *Alternaria* leaf spot symptoms appears as small brown to dark spots with concentric rings appears first on lower leaves and later spread on upper ones. These spots increases in number and size coalesce and form large irregular lesions. The center of mature spot usually becomes lighter in colour. Infected seed may show dark sunken lesions on the testa at the floret end. Seed may rot or seedling damping off and brown spots up to 5 mm in diameter appear on cotyledons. It has therefore become highly essential to have a good knowledge of such association, method of detection as well as way to control them. Considering economic importance of the disease, the present investigation was undertaken.

MATERIALS AND METHODS

In vitro efficacy of fungicides

Efficacy of eleven fungicides namely: mancozeb 75 WP, chlorothalonil 75 WP, copper oxychloride 50 WP, thiram 75 WP, captan 50 WP, difenconazole 25 EC, carbendazim 50 WP, hexaconazole 5 EC, propiconazole 25 EC, penconazole 10 EC, metalaxyl + mancozeb (Ridomil) 68 WP were evaluated (500, 1000, 1500 ppm each) *in vitro* against *A. carthami* by Poisoned food technique (Nene and Thapliyal, 1993). Based on active ingredient, the requisite quantity of each fungicide was calculated and mixed thoroughly with autoclaved and cooled (40°C) Potato Dextrose Agar medium (PDA) in conical flasks to obtain desired concentrations of 500, 1000 and 1500 ppm. Plain PDA medium without fungicides served as untreated control. Fungicide amended PDA medium was then poured aseptically in Petri plates (90 mm dia) and allowed to solidify at room temperature. After solidification of the medium, all the plates were inoculated aseptically with 5 mm culture disc of the test fungus obtained from a week old actively growing pure culture of *A. carthami*. The disc was placed on PDA in inverted position in the centre of the Petri plate and plates were incubated at 27 ± 1°C. Each treatment was replicated thrice. Observations on radial mycelial growth/colony diameter were recorded at 24 h interval and continued till the untreated control plate was fully covered with mycelial growth of the test fungus. Per cent mycelial growth inhibition of the test pathogen with the test fungicides over untreated control was calculated by applying the formula given by Vincent (1927).

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

Where, C = Growth (mm) of test fungus in untreated control plates
T = Growth (mm) of test fungus in treated plates

In vitro efficacy of bioagents

Six fungal antagonists namely: *Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T. koningii*, *T. lignorum, Gliocladium virens* and one bacterial antagonist namely: *Pseudomonas fluorescens* were evaluated *in vitro* against *A. carthami* applying Dual culture technique (Dennis and Webster, 1971). Seven days old cultures of the test bioagents and test fungus (*A. carthami*) grown on (PDA, NA) were used for the study. Discs (5 mm dia) of PDA along with culture growth of the test fungus and bioagents were cut out with sterilized cork borer. Then two culture discs, one each of the test fungus and bioagent were placed at equidistance and exactly opposite with each other on solidified PDA medium in Petri plates under aseptic conditions and plates were incubated at 27 ± 1°C PDA. The plates inoculated in centre only with culture disc of test fungus were maintained as untreated control and all the treatments were replicated thrice.

Observations on linear mycelial growth of the test fungus and bioagents were recorded at an interval of 24 h and continued till untreated control plates were fully covered with mycelial growth of the test fungus. Per cent inhibition of the test fungus over untreated control was calculated by applying the formula given by Arora and Upaddhay (1978).

$$\text{Per cent growth inhibition (PI)} = \frac{\text{Colony growth in control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

In vitro evaluation of botanicals (plant extracts)

Aqueous extracts of 11 botanicals namely: Mehandi (*Lawsonia innermis*), Ginger (*Zingiber officinale*), Dhatura (*Datura metal*), Tulsi (*Ocimum sanctum*), Parthenium (*Parthenium hysterophorus*), Neem (*Azadirachta indica*), Garlic (*Allium sativum*), Turmeric (*Curcuma longa*), Adulsa (*Adhotoda vasica*), Satawari (*Asparagus recemosus*) and Ghaneri (*Lantana camera*) were evaluated against *A. carthami*, applying poisoned food technique. Leaf extract of the test botanicals were prepared by grinding with mixture-cum grinder. The

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Table 1. *In vitro* efficacy of fungicides at different concentrations on mycelial growth and inhibition of *A. carthami*.

Treatment	Mean colony Dia (mm)* at Conc.			Av. Colony Dia. (mm)	% Inhibition at Conc.			Mean % inhibition
	500 ppm	1000 ppm	1500 ppm		500 ppm	1000 ppm	1500 ppm	
T_1 : Mancozeb	17.33	13.00	9.00	13.11	80.73 (53.85)	85.55 (58.82)	90.00 (64.17)	85.43(58.94)
T_2 : Chlorothalonil	68.33	65.33	59.66	64.44	24.07 (13.92)	27.40(15.90)	33.70 (19.69)	28.39 (16.50)
T_3: Copperoxy chloride	49.66	45.00	39.33	44.66	44.79 (26.62)	49.99 (30.00)	56.29 (34.26)	50.35 (30.29)
T_4 : Thiram	19.66	14.66	10.66	14.99	78.14 (51.40)	83.70 (56.84)	88.15 (61.85)	83.33 (56.69)
T_5 : Captan	46.00	41.33	37.66	41.66	48.88 (29.26)	54.07 (32.73)	58.15 (35.54)	53.70 (32.51)
T_6 : Difenconazole	23.16	20.50	18.00	20.55	74.26 (47.95)	72.22 (50.54)	80.00 (52.07)	77.16 (50.18)
T_7 : Carbendazim	05.00	05.00	5.00	05.00	94.44 (70.79)	94.44 (70.79)	94.44 (70.79)	94.44 (70.79)
T_8 : Hexaconazole	21.00	14.16	10.83	15.33	76.66 (50.07)	84.25 (57.41)	87.96 (61.59)	82.95 (56.35)
T_9 : Propiconazole	24.83	21.83	18.33	21.66	72.40 (46.38)	75.74 (49.23)	79.63 (52.77)	75.92 (49.46)
T_{10} : Penconazole	25.16	22.05	18.66	22.10	72.03 (46.08)	75.00 (48.58)	79.26 (52.41)	75.43 (49.02)
T_{11} :Ridomil-MZ	19.33	15.01	11.83	15.42	78.51 (51.74)	83.14 (56.25)	86.85 (60.29)	82.83 (56.09)
T_{12} : Control (Untreated)	90.00	90.00	90.00	90.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
S.E. ±	1.41	0.67	0.70	0.92	0.74	0.63	0.70	0.69
CD (P=0.05)	4.12	1.95	2.04	2.70	2.17	1.83	2.04	2.01

*: Means of three replications Figures in parenthesis are arc sine transformed value.

100 g washed leaves of each botanical, ginger rhizome and garlic bulbs (100 g each) were macerated in 100 ml distilled water (w/v) separately and the macerate obtained were filtered through double layered muslin cloth. Each of the filtrate obtained was further filtered through Whatman No. I filter paper using funnel and volumetric flasks (100 ml cap.). The final clear extracts/filtrates obtained formed the standard plant extracts of 100% concentration. These were evaluated (at 10, 15 and 20% each) *in vitro* against *A. carthami*, applying poisoned food technique (Nene and Thapliyal, 1993) and using potato dextrose agar (PDA) as basal culture medium.

An appropriate quantity of each plant extract (100%) was separately mixed thoroughly with autoclaved and cooled (40°C) PDA medium in conical flasks (250 ml cap) to obtain desired concentrations (10,15 and 20%). Sterilized and cooled PDA medium amended separately with plant extract was then poured (15 to 20 ml/plate) into sterile glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. Each plant extract and its respective concentrations were replicated thrice. The plates containing PDA without any plant extract were maintained as untreated control. Upon solidification of PDA, all the treatment 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. carthami*. Plates containing plain PDA and inoculated with mycelial disc of test fungus served as untreated control. All these plates were then incubated at 27 $\pm$ 1°C temperature for a week or till the untreated control plates were fully covered with mycelial growth of the test fungus.

Observations on radial mycelial growth/colony diameter of the test fungus were recorded treatment wise at 24 h interval and continued till mycelial growth of the test fungus was fully covered in the untreated control plates. Per cent inhibition of mycelial growth over untreated control was calculated by applying the formula given by Vincent (1927).

RESULTS AND DISCUSSION

In vitro evaluation of fungicides

A total of eleven fungicides namely: mancozeb 75 WP, chlorothalonil 75 WP, copper oxychloride 50 WP, thiram 75 WP, captan 50 WP, difenconazole 25 EC, carbendazim 50 WP, hexaconazole 5 EC, propiconazole 25 EC, penconazole 10 EC and metalaxyl + mancozeb (Ridomil) 68 WP were evaluated (at 500, 1000 and 1500 ppm each) *in vitro* against *A. carthami*, applying poisoned food technique and using potato dextrose agar (PDA) as basal medium. Effect of these fungicides on radial mycelial growth and inhibition of the test pathogen over untreated control were recorded and the results obtained are presented in Table 1, Plate-I A B C, and Figures 1, 2 and 3.

Mycelial growth/colony diameter

Result (Table 1) revealed that all the fungicides tested covered a wide range of radial mycelial growth/colony diameter of the test pathogen, depending upon their concentrations used (Plate I).

At 500 ppm, radial mycelial growth of pathogen was ranged from 5.00 (carbendazim) to 68.33 (chlorothalonil) mm. However, it was the maximum with chlorothalonil (68.33 mm) and this was followed by copper oxychloride (49.66 mm), Captan (46.00 mm), both of which were at par. Significantly least mycelial growth was recorded with carbendazim (5.00 mm) followed by mancozeb (17.33 mm), ridomil (19.33 mm), hexaconazole (21.00 mm), all three of which were at par. difenconazole (23.16 mm), propiconazole (24.83 mm) and penconazole (25.16 mm) all three of which were at par (Plate I (A) and Figure 1).

At 1000 ppm, radial mycelial growth of the test pathogen was ranged from 5.00 mm (carbendazim) to 65.33 mm (chlorothalonil). All the fungicides tasted exhibited similar trends of mycelial growth as that of 500 ppm. However, it was maximum with chlorothalonil (65.33 mm). This was followed by copper oxychloride (45.00

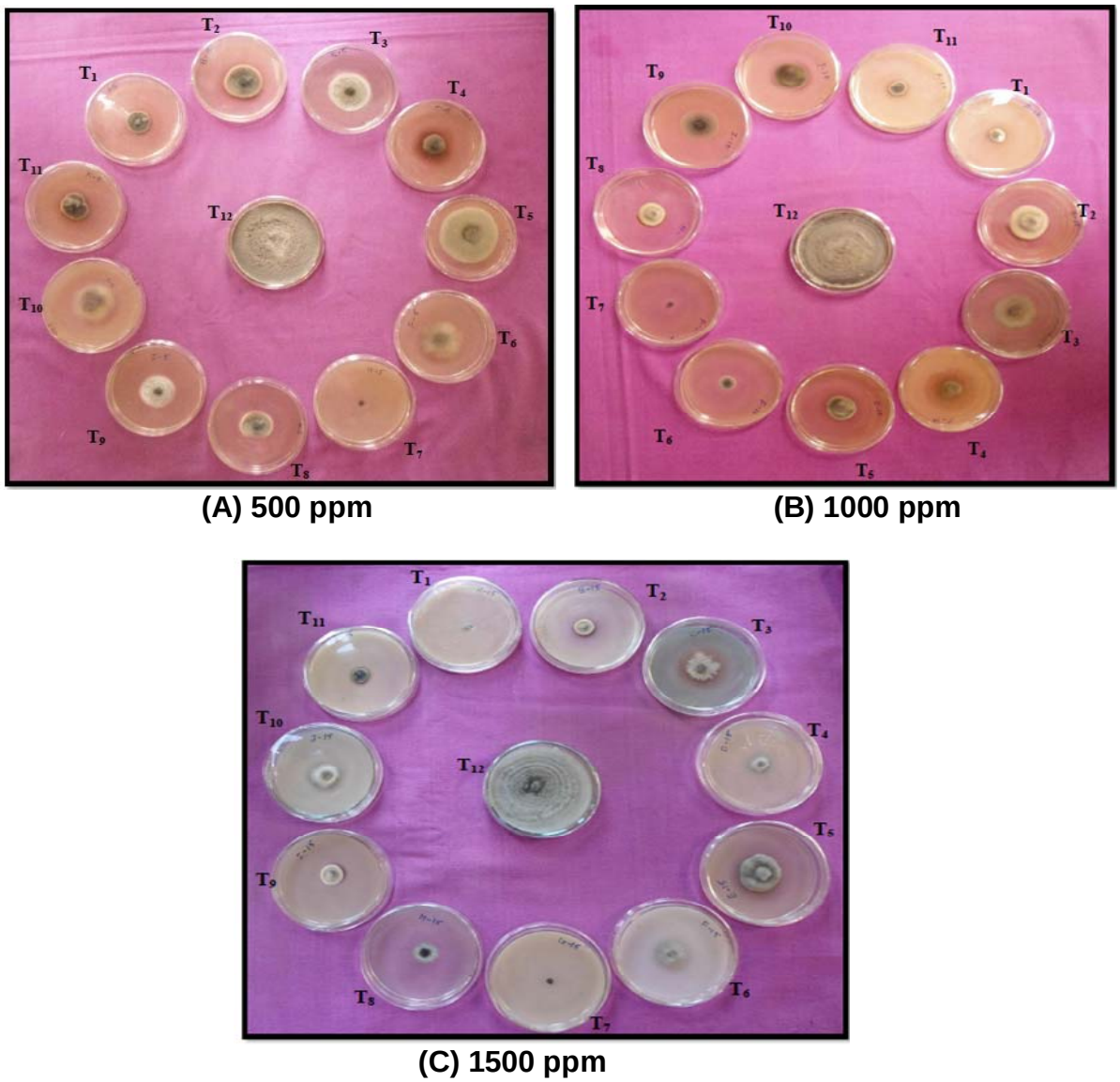

Plate I. *In vitro* efficacy of fungicides at 500 ppm (A), 1000 ppm (B) and 1500 ppm concentration (C) on radial mycelial growth and inhibition of *A. carthami.*

mm), Captan (41.33 mm). Significantly least mycelial growth was recorded with the carbendazim (5.00 mm), followed by mancozeb (13.00 mm), hexaconazole (14.16 mm), thiram (14.66 mm), ridomil (15.01 mm) all which were at par and difenconazole (20.50 mm), propiconazole (21.83 mm), penconazole (22.05 mm) all which were at par (Plate I (B) and Figure 2).

At 1500 ppm, radial mycelial growth of the test pathogen was ranged from 5.00 mm (carbendazim) to 59.66 mm (chlorothalonil). However, it was maximum with chlorothalonil (59.66 mm) and this was followed by copper oxychloride (39.33 mm), captan (37.66 mm) both of which were at par. Significantly minimum mycelial growth was recorded at with the fungicides namely: carbendazim (5.00 mm) followed by mancozeb (9.00 mm), thiram (10.66 mm), hexaconazole (10.83 mm), ridomil (11.83 mm) all which were at par and difenconazole (18.00 mm), propiconazole (18.33 mm), penconazole (18.66 mm) all which were at par. (Plate I (C) and Figure 3).

Average radial mycelial growth recorded (Table 1) with the fungicides tested (at 500, 1000 and 1500 ppm each) was ranged from 5.00 (carbendazim) to 64.44 mm (chlorothalonil). However, highest mean radial mycelial growth was recorded with chlorothalonil (64.44 mm). This was followed by copper oxychloride (44.66 mm) and captan (41.66 mm). Comparatively minimum average mycelial growth was recorded with the fungicides *viz*., Carbendazim (5.00 mm), followed by Mancozeb (13.11 mm), Thiram (14.99 mm), Hexaconazole (15.33 mm), Ridomil (15.42 mm), difenconazole (20.55 mm), propiconazole

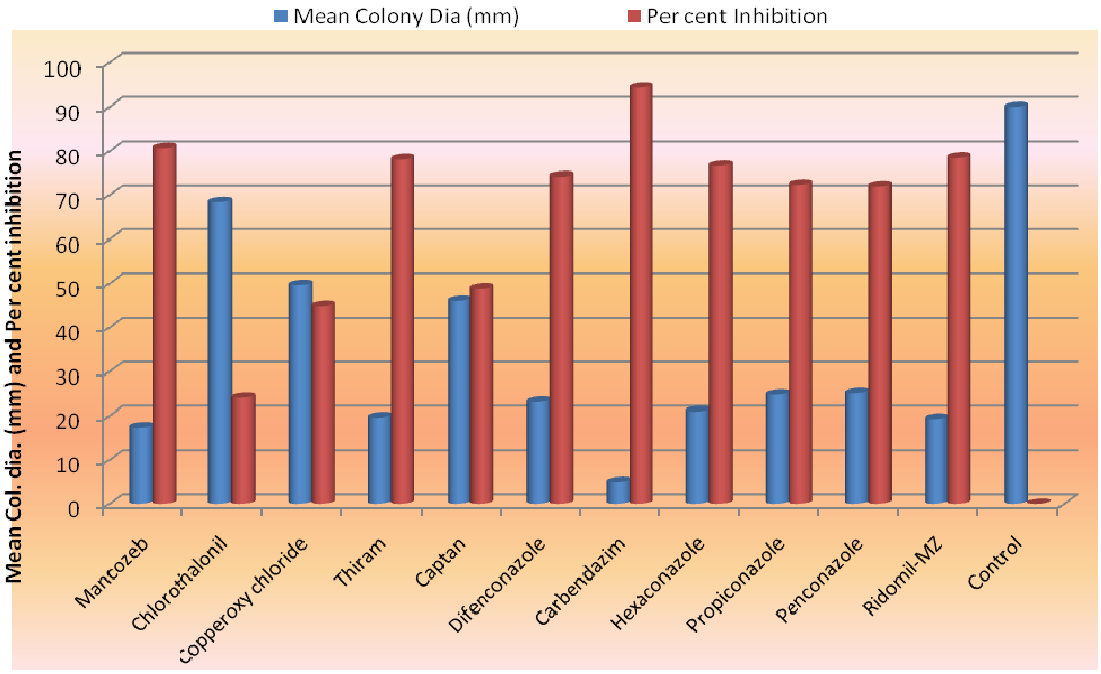

Figure 1. *In vitro* efficacy of fungicide at 500 ppm concentration on mycelia growth and inhibition of *A. carthami.*

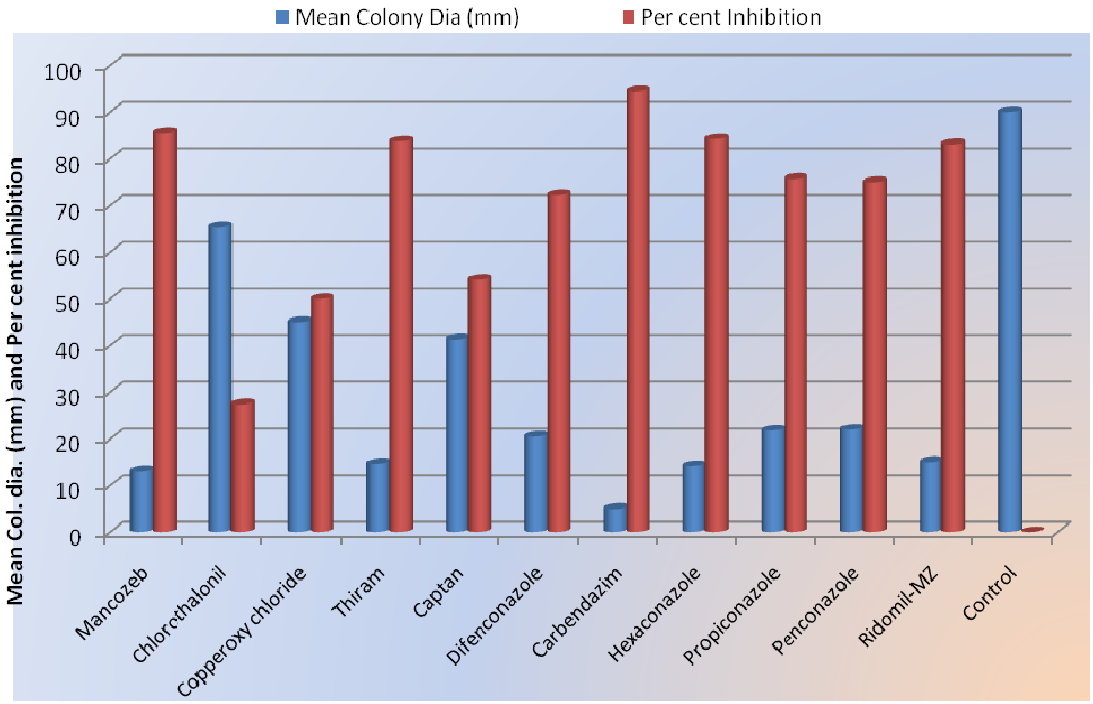

Figure 2. *In vitro* efficacy of fungicide at 1000 ppm concentration on mycelia growth and inhibition of *A. carthami.*

(21.66 mm), penconazole (22.10 mm) as compared to 90.00 mm average growth of the pathogen in untreated control.

Mycelial growth inhibition

Results (Table 1, Plate I, and Figures 1, 2, 3) revealed

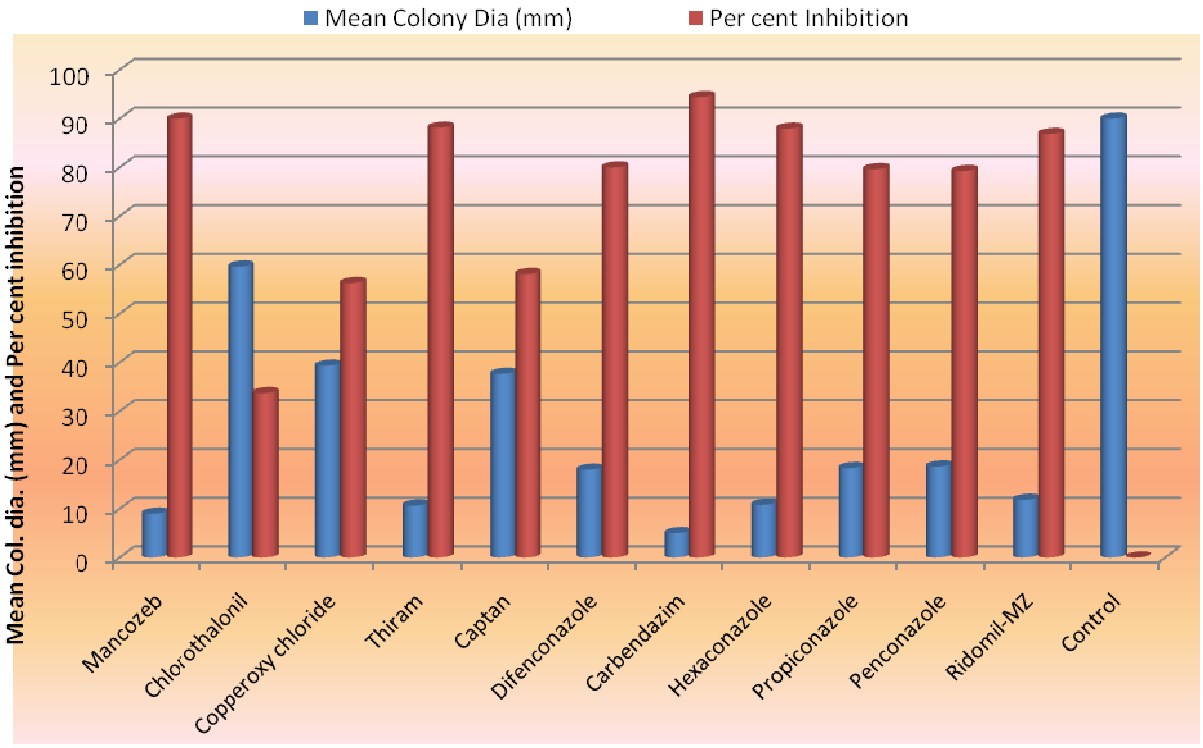

Figure 3. *In vitro* efficacy of fungicide at 1500 ppm concentration on mycelia growth and inhibition of *A. carthami.*

that all the fungicides tested (at 500, 1000 and 1500 ppm each) significantly inhibited mycelial growth of the test pathogen over untreated control (0.00%). Further, it was found that percentage mycelial inhibition of test pathogen was increased with increase in concentrations of the fungicides tested.

At 500 ppm, percentage mycelial growth inhibition (Plate I A) was ranged from 24.07 (chlorothalonil) to 94.44 (carbendazim) over untreated control (0.00) per cent. However, significantly highest mycelial growth inhibition was recorded with carbendazim (94.44%). This was followed by mancozeb (80.73%), ridomil (78.51%), thiram (78.14%), hexaconazole (76.66%), difenconazole (74.26%), propiconazole (72.40%) and penconazole (72.03%). The fungicides chlorothalonil, copper oxychloride and captan were found least effective and recorded significantly minimum mycelial inhibition, 24.07, 44.79 and 48.88%, respectively.

At 1000 ppm, percentage of mycelial growth inhibition (Plate I B) was ranged from 27.40% (chlorothalonil) to 94.44% (carbendazim) over untreated control (0.00%). However, significantly highest mycelial growth inhibition was recorded with carbendazim (94.44%). This was followed by fungicidesnamely: mancozeb (85.5%), hexaconazole (84.25%), thiram (83.70%), ridomil (83.14%), propiconazole (75.74%), penconazole (75.00%) and difenconazole (72.22%) comparatively minimum inhibition was recorded with chlorothalonil (27.40%), copper oxychloride (49.99%) and captan (54.07%).

At 1500 ppm, similar trend of mycelial growth inhibition (Plate I C) with test fungicides was recorded as that of 500 and 1000 ppm and it was ranged from 33.70% (chlorothalonil) to 94.44% (carbendazim) over untreated control (0.00%). However, significantly highest mycelial inhibition was recorded with carbendazim (94.44%). The second and third best fungicides were found mancozeb (90.00%) and thiram (88.15%). This was followed by fungicides namely: hexaconazole (87.96%), ridomil (86.85%), difenconazole (80.00%), propiconazole (79.63%), penconazole (79.26%) and captan (58.15%). Whereas, chlorothalonil and copper oxychloride was found comparatively least effective and recorded minimum inhibition 33.70 and 56.29%, respectively.

Mean percentage mycelial growth inhibition recorded with all fungicides tested (at 500, 1000 and 1500 ppm each) was ranged from 28.39% (chlorothalonil) to 94.44% (carbendazim). However, carbendazim and mancozeb were found most fungistatic each of which recorded significantly highest mean mycelial growth inhibition of 94.44% and 85.43%, respectively over untreated control (0.00%). This was followed by thiram (83.33%), hexaconazole (82.95%), ridomil (82.83%), difenconazole (77.16%), propiconazole (75.92%) and penconazole (75.43%). Less than 51% mean growth inhibition was recorded with chlorothalonil (28.39%) and copper oxychloride (50.35%).

Thus, all the fungicides tested were found fungistatic against the test pathogen *A. carthami* and significantly inhibited its mycelial growth over untreated control.

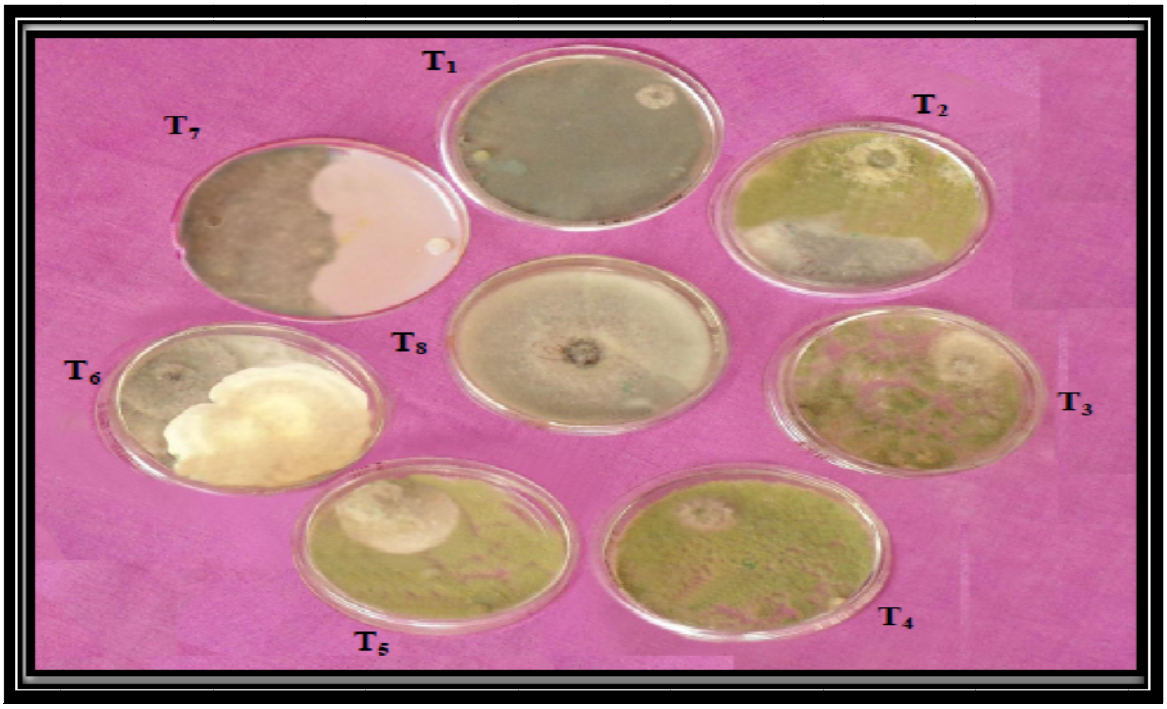

Plate II. *In vitro* efficacy of bioagents on mycelial growth and inhibition of *A. carthami* .

However, carbendazim recorded highest mean mycelial growth inhibition (94.44%), second and third best fungicides were mancozeb (85.83%) and thiram (83.33%). This was followed by hexaconazole (82.95%), ridomil (82.83%),difenconazole(77.16%),propiconazole(75.92%), and penconazole (75.43%). Fungicides chlorothalonil and copper oxychloride were found least effective, which recorded minimum mean mycelial inhibition of 28.39 and 50.35%, respectively. Similar fungistatic effect of the fungicides against *A. carthami* infecting safflower and *Alternaria* spp. infecting many other crops were reported earlier by several workers. (Krishna et al., 1998; Bramhankar et al., 2001; Amaresh and Nargund, 2002; Ambhore et al., 2005; Mesta et al., 2009; Murumkar et al., 2009).

In vitro evaluation of bioagents

Six fungal (*Trichoderma viride, T. harzianum, T. koningii, T. hamatum, T. lignorum* and *Gliocladium virens*) and one bacterial (*Pseudomonas fluorescens*) bioagents/antagonists were evaluated *in vitro* against *A. carthami*, applying dual culture technique and using PDA as basal medium. The results obtained mycelial growth and inhibi-tion are presented in Plate II, Table 2 and Figure 4.

Results (Table 2) revealed that all the bioagents/antagonists evaluated exhibited fungistatic/antifungal activity and significantly inhibited mycelial growth of *A. carthami* over untreated control (Plate II). Amongst the seven bioagents tested, *T. viride* was found most effective which recorded significantly least linear mycelial growth (5.33 mm) and corresponding highest mycelial growth inhibition (94.07%) of the test pathogen over untreated control (90.00 mm and 0.00%, respectively). The second and third best bioagents/antagonists found were *T. hamatum* and *T. koningii* which recorded mycelial growth of 13.33 and 17.00 mm, respectively and inhibition of 85.18 and 81.11%, respectively. This was followed by *T. lignorum*, *T. harzianum* and *Gliocladium virens* (growth: 23.33, 34.66 and 41.66 mm, and inhibition: 74.07, 61.48 and 53.71%, respectively). The bacterial antagonists *Pseudomonas fluorescens* was found comparatively least effective and recorded mycelial growth of 45.66 mm and inhibition of 49.26% of the test pathogen.

Thus, all the bioagents evaluated *in vitro* were found fungistatic/antifungal against *A. carthami* and caused significant reduction in mycelial growth of the test pathogen over untreated control. The inhibitory of the *Trichoderma* spp. and *P. fluorescens* against *A. carthami* may be attributed to the mechanisms namely: antibiosis, lysis, mycoparasitism, competition and production of volatile substances.

Results of the present study on antagonistic effects of the *Trichoderma* spp. and *P. fluorescens* against *Alternaria* spp. are in conformity with those reported earlier by several workers (Ghosh et al., 2002; Singh et al., 2005; Mishra and Gupta, 2008).

In vitro efficacy of plant extracts/botanicals

A total of 11 plant extracts/botanicals namely: Mehandi (*Lawsonia innermis*), Ginger (*Zingiber officinale*), Dhatura (*Datura metal*), Tulsi (*Osmium sanctum*), Parthenium

Table 2. *In vitro* efficacy of different bioagents on mycelial growth and inhibition of *A. carthami*.

Treatment	Mean colony Dia(mm)* of pathogen	Inhibition (%)
T_1 : *Trichoderma viride*	5.33	94.07 (70.17)
T_2 : *T. harzianum*	34.66	61.48 (37.34)
T_3: *T. koningii*	17.00	81.11 (54.43)
T_4 : *T. hamatum*	13.33	85.18 (58.43)
T_5 : *T. lignorum*	23.33	74.07 (47.79)
T_6 : *Gliocladium virens*	41.66	53.71 (32.48)
T_7 : *Pseudomonas fluorescens*	45.66	49.26 (29.50)
T_8 : Control (Untreated)	90.00	00.00 (00.00)
SE ±	**0.84**	**0.82**
C.D. (P = 0.05)	**2.51**	**2.46**

*Mean of three replications. Figures in parenthesis are arc sine transformed value.

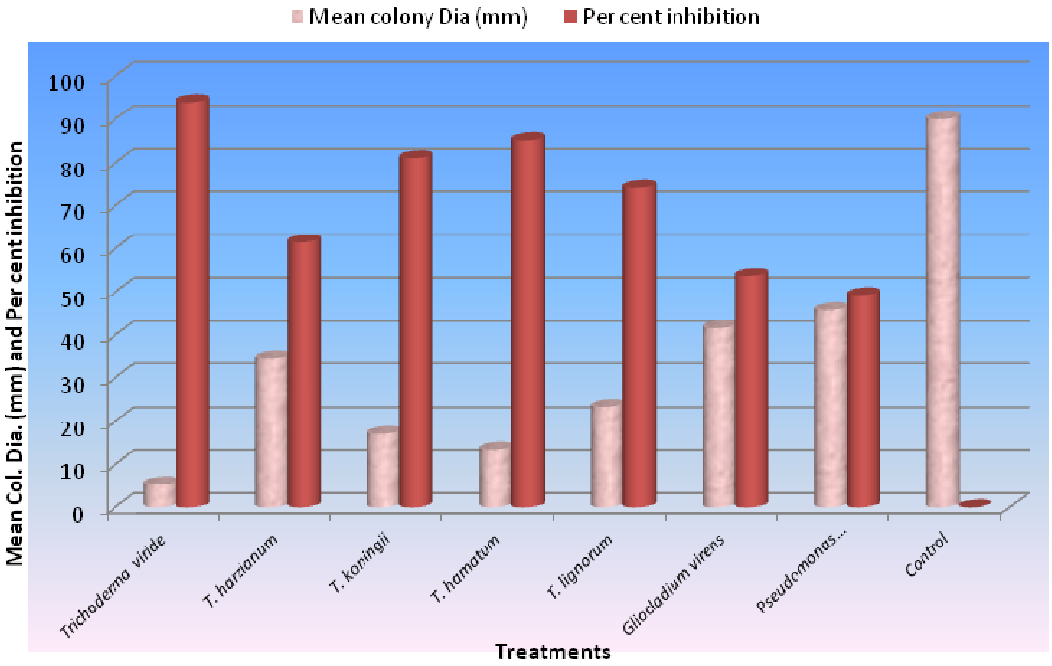

Figure 4. *In vitro* efficacy of different bioagents on mycelial growth and inhibition of *A. carthami*

(*Parthenium hysterophorus*), Neem (*Azadirachta indica*), Garlic (*Allium sativum*), Turmeric (*Curcuma longa*), Adulsa (*Adhotoda vasica*), Satawari (*Asparagus recemosus*) and Ghaneri (*Lantana camera*) were evaluated (at 10, 15 and 20% each) *in vitro* against *A. carthami*, applying poisoned food technique and using PDA as a basal medium. Results obtained on the effects of the test botanicals/phytoextracts on radial mycelial growth and inhibition of the test pathogen over untreated control were recorded and presented in the Table 3, Plate III A, B, C and Figures 5, 6, 7.

Radial mycelial growth

Results (Table 3, Plate III) revealed that all the botanicals /plant extracts tested exhibited a wide range of radial mycelial growth of test pathogen, depending up on their concentration used and it was decreased with increase in concentration of the botanicals tested.

At 10%, radial mycelial growth of the test pathogen was ranged from 36.66 (*A. sativum*) to 75.33 (*A. racemosus*) mm. However, it was maximum with *A. racemosus* (75.33 mm). This was followed by *O. sanctum* (74.66 mm), *P. hysterophorus* (71.66 mm), *L. innermis* (69.33 mm), *A. indica* (68.33 mm), three of which were at par and *Z. officinale* (64.33 mm), *L. camera* (60.66 mm), *A. vasica* (55.66 mm), *C. longa* (51.33 mm) of which were at par. comparatively least mycelial growth was recorded with the botanicals *viz*., *A. sativum* (36.66 mm) and *D. metal* (47.66 mm) Plate III A, Figure 5.

Table 3. *In vitro* efficacy of different plant extracts/botanicals on mycelial growth and inhibition of *A. carthami*.

Treatment	Colony Dia. Mean (mm) * at Conc.			Mean (mm)	% Inhibition at Conc.			Mean % Inhibition
	10 %	15 %	20 %		10 %	15 %	20 %	
T_1 : Mehandi (*L. innermis*)	69.33	64.66	58.33	64.10	22.96 (13.27)	28.15 (16.34)	35.18 (20.59)	28.76 (16.73)
T_2 : Ginger (*Z. officinale*)	64.33	58.33	56.33	59.66	(28.52 (16.56)	35.18 (20.59)	37.41 (21.96)	33.70 (19.70)
T_3: Dhatura (*D. metal*)	47.66	44.66	43.00	45.10	47.04 (28.05)	50.37 (30.24)	52.22 (31.47)	49.87 (29.83)
T_4 : Tulasi (*O. sanctum*)	74.66	72.33	70.00	72.33	17.04 (10.01)	19.63 (11.31)	22.22 (12.83)	19.63 (11.38)
T_5 : Parthenium (*P. hysterophorus*)	71.66	67.33	63.00	67.33	20.37 (22.53)	25.18 (14.58)	30.00 (17.45)	25.18 (18.18)
T_6 : Neem (*A. indica*)	68.33	66.33	61.00	65.22	24.07 (13.92)	26.30 (23.93)	32.22 (18.79)	27.53 (18.88)
T_7 : Garlic (*A. sativum*)	36.66	34.00	30.66	33.77	59.26 (35.28)	62.22 (37.66)	65.93 (41.23)	62.47 (38.05)
T_8 : Turmeric (*C. longa*)	51.33	47.33	44.66	47.77	42.96 (25.44)	47.41 (28.29)	50.37 (30.24)	46.91 (27.99)
T_9 : Adulsa (*A. vasica*)	55.66	53.66	52.00	53.77	38.15 (22.42)	40.37 (23.80)	42.22 (24.97)	40.24 (23.73)
T_{10} : Satawari (*A. racemosus*)	75.33	73.33	69.00	72.66	15.93 (9.16)	18.52 (10.52)	23.33 (13.49)	19.26 (11.06)
T_{11} : Ghaneri (*L. camera*)	60.66	57.66	53.33	57.21	32.60 (19.01)	35.93 (21.04)	40.74 (24.03)	36.42 (21.36)
T_{12} : Control (Untreated)	90.00	90.00	90.00	90.00	00.00 (00.00)	0.00 (0.00)	00.00 (0.00)	00.00 (00.00)
S.E. ±	0.79	0.63	0.75	0.72	0.87	0.69	0.52	0.69
CD (P=0.05)	2.31	1.83	2.20	2.11	2.61	2.08	1.53	2.07

*: Means of three replications. Figures in parenthesis are arc sine transformed value.

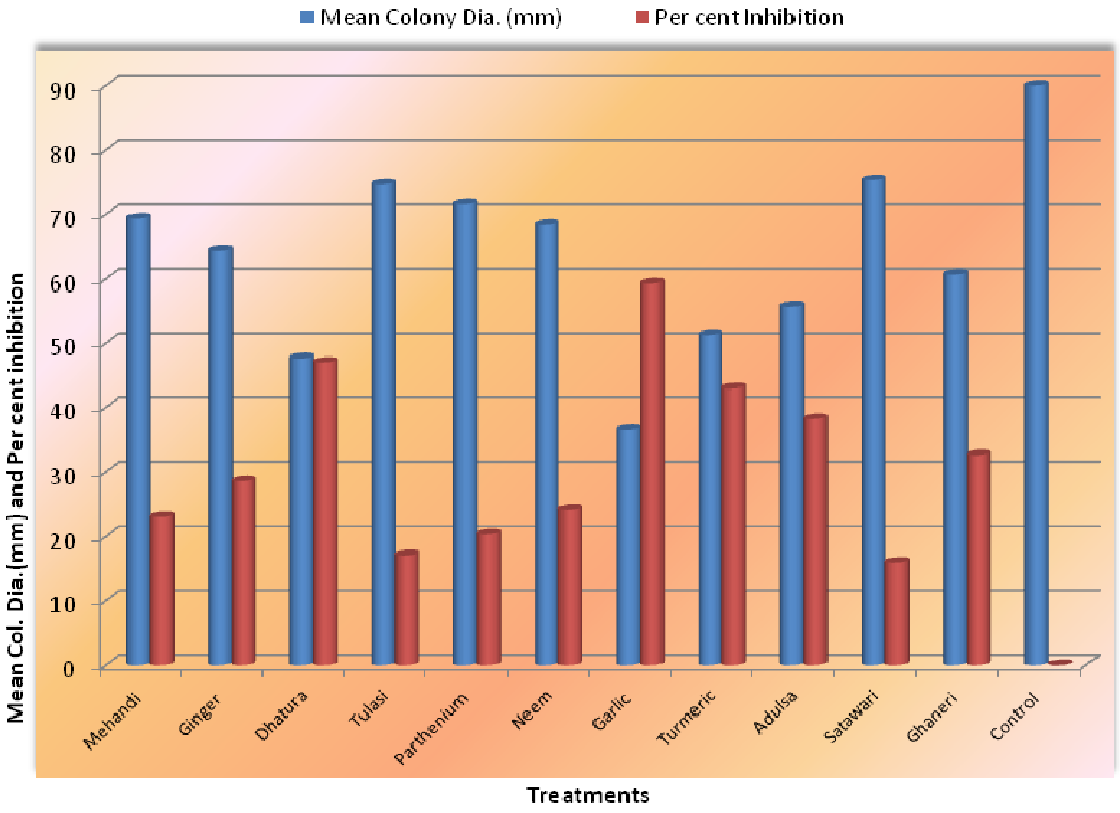

Figure 5. *In vitro* efficacy of 10% plant extracts on mycelial growth and inhibition of *A. carthami*.

At 15 per cent, radial mycelial growth of the test pathogen was ranged from 34.00 (*A. sativum*) to 73.33 (*A. racemosus*) mm. However, it was maximum with *A. racemosus* (73.33 mm). This was followed by *O. sanctum* (72.33 mm) both of which were at par and *P. hysterophorus* (67.33 mm), *A. indica* (66.33 mm), *L. innermis* (64.66 mm). All of which were at par to each other and *Z. officinale* (58.33 mm), *L. camera* (57.66 mm), *A. vasica* (53.66 mm)

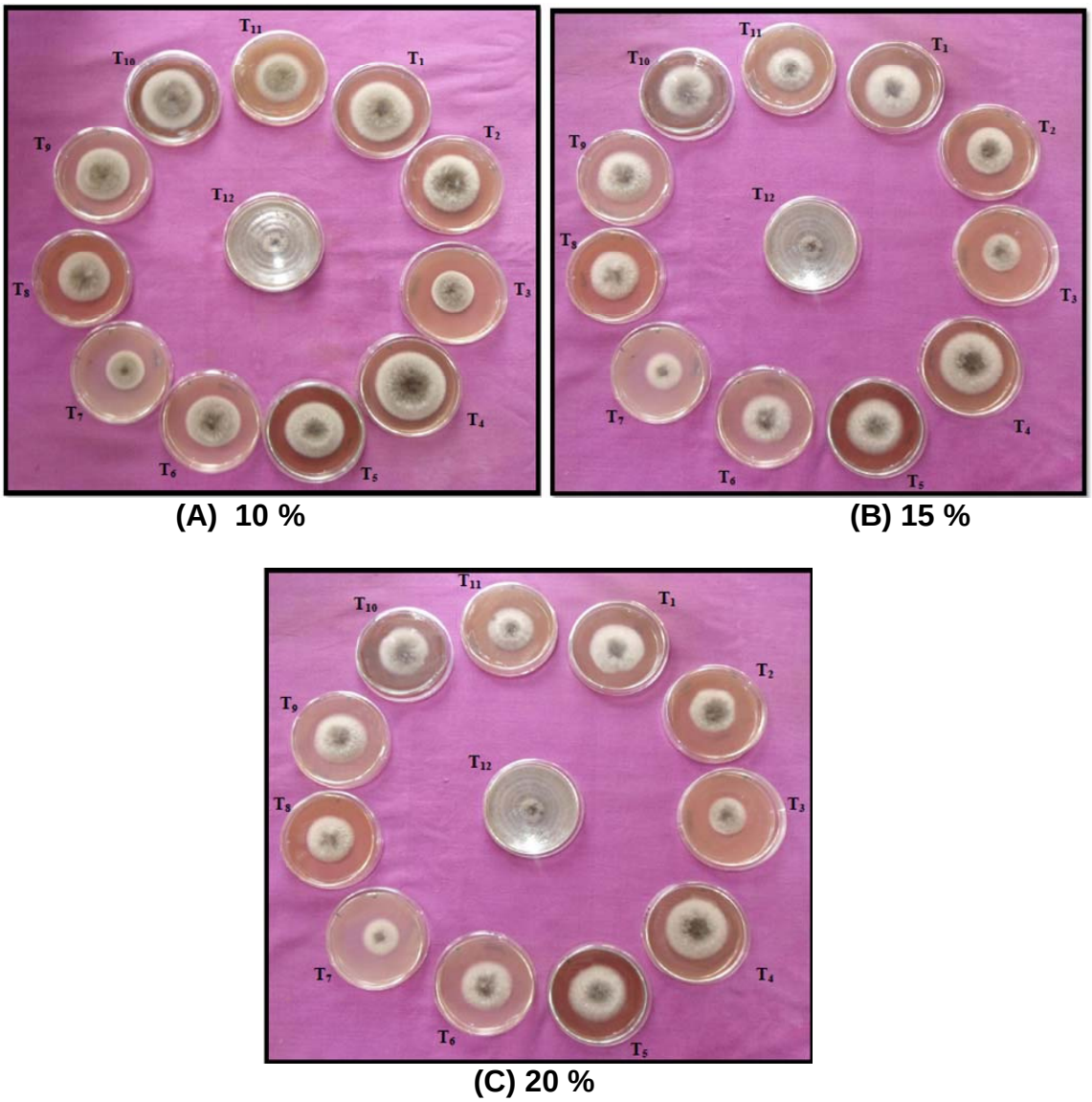

Plate III. *In vitro* efficacy of plant extract/botanicals at 10% (A), 15% (B) and 20% (C) on growth and inhibition *A. carthami*.

of which were at par. Significantly least mycelial growth was recorded with the botanicals *A. sativum* (34.00 mm), *D. metal* (44.66 mm) and *C. longa* (47.33 mm) (Plate III B, Figure 6).

At 20%, radial mycelial growth of the test pathogen was ranged from 30.66 mm (*A. sativum*) to 70.00 mm (*O. sanctum*). However, significantly highest mycelial growth was maximum with *O. sanctum* (70.00 mm). This was followed by *A. racemosus* (69.00 mm) both of which were at par and *P. hysterophorus* (63.00 mm), *A. indica* (61.00 mm), *L. innermis* (58.33 mm), *Z. officinale* (56.33 mm). All of which were at par to each other and L. camera (53.33 mm), *A. vasica* (52.00 mm), both of which were at par. Less than 50 mm growth was recorded with the botanicals *C. longa* (44.66 mm) and *D. metal* (43.00 mm) both of which were at par. Significantly least mycelial growth was recorded with the botanical *A. sativum* (30.66 mm), as compared to untreated control was 90.00 mm (Plate III C, Figure 7).

The mean percentage radial mycelial growth recorded with the plant extracts tested (at 10, 15 and 20% each) was ranged from 33.77 mm (*A. sativum*) to 72.66 mm (*A. racemosus*). However, significantly highest mean mycelial growth was recorded with *A. racemosus* (72.66 mm). This was followed by O. sanctum (72.33 mm) both of which were at par and *P. hysterophorus* (67.33 mm), *A. indica* (65.22 mm), *L. innermis* (64.10 mm), *Z. officinale* (59.66 mm), *L. camera* (57.21 mm). Comparatively less mycelial growth was recorded with the botanicals *A. vasica* (53.77 mm), *C. longa* (47.77 mm) and *D. metal*

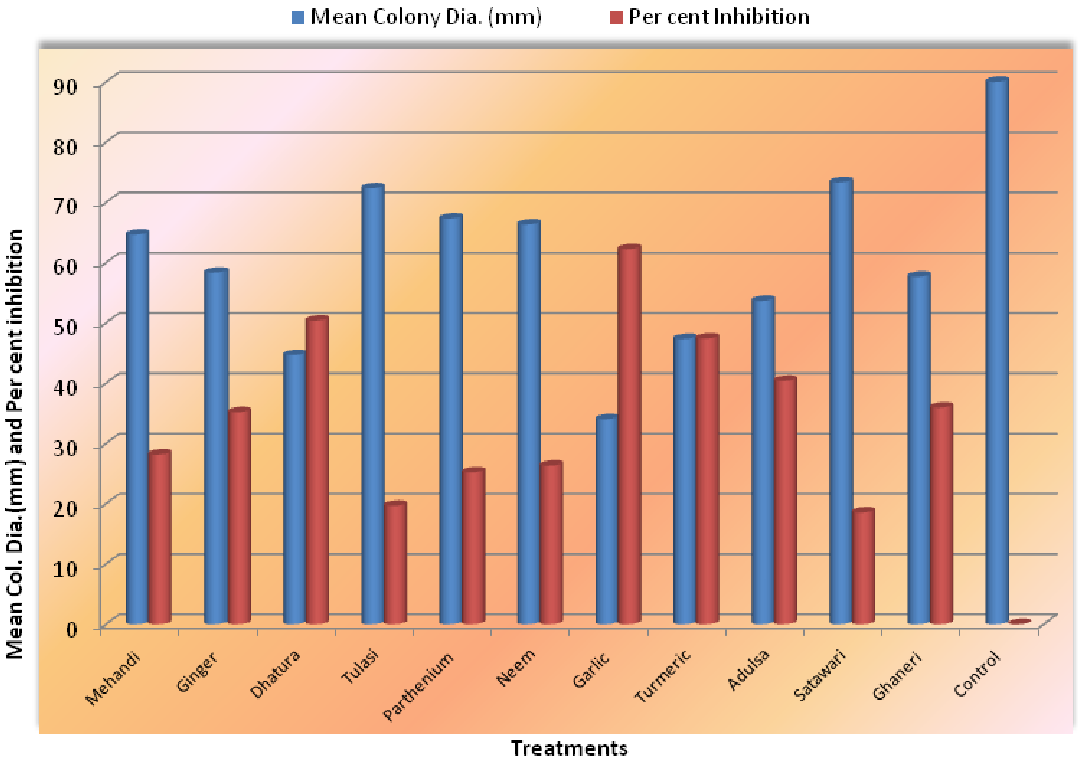

Figure 6. *In vitro* efficacy of 15% plant extracts on mycelial growth and inhibition of *A.carthami* .

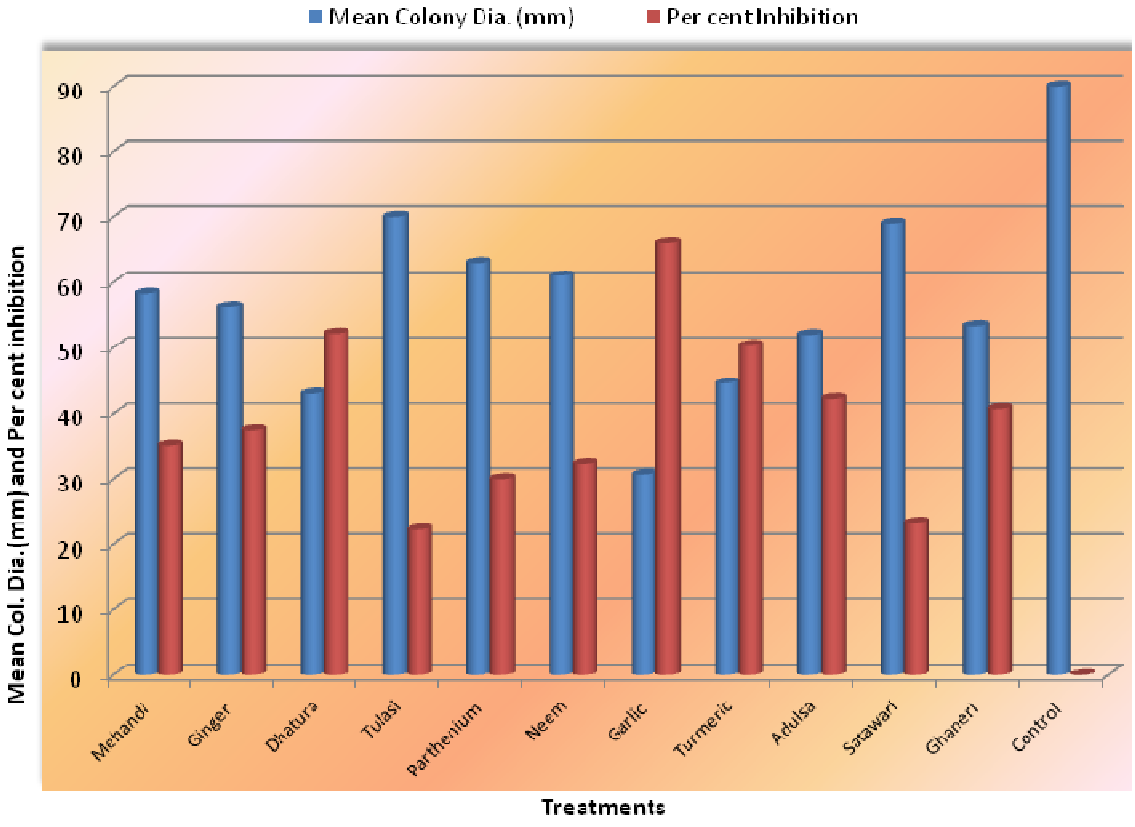

Figure 7. *In vitro* efficacy of 20% plant extracts on mycelial growth and inhibition of *A.carthami* .

(45.10 mm). Significantly least mean mycelial growth was recorded with *A. sativum* (33.77 mm). This was followed by *D. metal* (45.10 mm). The mean mycelial growth was recorded in untreated control was 90.00 mm.

Mycelial growth inhibition

Results (Table 3, Plate III, A, B, C, and Figures 5, 6, 7) revealed that all the plant extracts tested (at 10, 15 and 20% each) significantly inhibited mycelial growth of the test pathogen over untreated control (0.00%). Further, it was found that per cent mycelial growth inhibition of the test pathogen was increased with increase in concentration of the botanicals tested.

At 10%, mycelial growth inhibition was ranged from 15.93 (*A. racemosus*) to 59.26 (*A. sativum*) per cent. However, significantly highest mycelial growth inhibition was recorded with *A. sativum* (59.26%). This was followed by *D. metal* (47.04%), *C. longa* (42.96%), *A. vasica* (38.15%) and L. camera (32.60%). Comparatively less mycelial growth inhibition was recorded with *Z. officinale* (28.52%), *A. indica* (24.07%) and *L. innermis* (22.96%) both of which were at par. Significantly least mycelial growth inhibition was recorded with botanicals namely: *A. racemosus* (15.93%). This was followed by *O. sanctum* (17.04%) and *P. hysterophorus* (20.37%) over untreated control (0.00%).

At 15%, mycelial growth inhibition was ranged from 18.52 (*A. racemosus*) to 62.22 (*A. sativum*) per cent. However, significantly highest mycelial growth inhibition was recorded with *A. sativum* (62.22%). This was followed by *D. metal* (50.37%), *C. longa* (47.41%) and *A. vasica* (40.37%). Comparatively less mycelial growth inhibition was recorded with *L. camera* (35.93%) and *Z. officinale* (35.18%) both of which were at par and *L. innermis* (28.15%), *P. hysterophorus* (25.18%) and *O. sanctum* (19.63%). Significantly least mycelial growth inhibition was recorded with *A. racemosus* (18.52%) over untreated control (0.00%).

At 20%, mycelial growth inhibition was ranged from 22.22 (*O. sanctum*) to 65.93 (*A. sativum*) per cent. However, significantly highest mycelial growth inhibition was recorded with *A. sativum* (65.93%). This was followed by *D. metal* (52.22%), *C. longa* (50.37%) both of which were at par and *A. vasica* (42.22%), *L. camera* (40.74%) both of which were at par. Comparatively less mycelial growth inhibition was recorded with *Z. officinale* (37.41%), *L. innermis* (35.18%), *A. indica* (32.22%) and *P. hysterophorus* (30.00%). Significantly least mycelial growth inhibition was recorded with *A. racemosus* (23.33%) and *O. sanctum* (22.22%) over untreated control (0.00%).

Mean percentage mycelial growth inhibition recorded with all the test botanicals was ranged from 19.26 (*A. racemosus*) to 62.47 (*A. sativum*) per cent. However, *A. sativum* was found most fungistatic and recorded significantly highest mean mycelial growth inhibition (62.47%). The second and third best plant extracts found were *D. metal* (49.87%) and *C. longa* (46.91%). This was followed by *A. vasica* (40.24%), *L. camera* (36.42%), *Z. officinale* (33.70%) and *L. innermis* (28.76%). Comparatively minimum mycelial inhibition was recorded with *A. indica* (27.53%), *P. hysterophorus* (25.18%), *O. sanctum* (19.63%) and *A. racemosus* (19.26%) over untreated control (0.00%).

Thus, all the plant extracts tested were found fungistatic against *A. carthami* and significantly inhibited its mycelial growth over untreated control. However, *A. sativum* recorded highest mean mycelial growth inhibition (62.47%) and this was followed by *D. metal* (49.87%) and *C. longa* (46.91%).

Thus, all the botanicals/plant extracts evaluated *in vitro* were found fungistatic and significantly inhibited mycelial growth of *A. carthami*. The fungistatic effects of the botanicals might be due to the presence of specific antifungal chemical compounds like phenols, tannis, alkaloids, resinous and non volatile substances.

Similar results of antifungal/fungistatic effect of botanicals/plant extracts against *A. carthami* and *Alternaria* spp. were reported earlier by several workers (Singh and Mujumdar, 2001; Ghosh et al., 2002; Shinde et al., 2008; Mesta et al., 2009; Ranaware et al., 2010).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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