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Efficacy of Fungicides, Bioagents and Phytoextracts against *Alternaria carthami* of Safflower in *In Vitro* Condition

S.S. Wagh, A.P. Suryawanshi and D.V. Pawar

Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani-431402 (M.S.), India.

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Safflower leaf spot / blight caused by *Alternaria carthami* (Chowdhury) is serious diseases in India. Considering the economic importance and seriousness of the disease, the present cost effective management practices against the test pathogen present *in vitro* studies were conducted during 2012-13. All the experiments were planned with CRD and all the treatments replicated thrice. A total six systemic fungicides @ 500, 1000 and 1500 ppm concentration and seven non-systemic / combi fungicides @ 1000, 2000 and 2500 ppm concentrations was evaluated *in vitro* against *A. carthami*, applying Poisoned food technique (Nene and Thapliyal, 1993). Results of the present studies revealed that systemic fungicides most effective than non-systemic against the test pathogen. Among systemic fungicides, average cent per cent inhibition of mycelial growth was observed in Hexaconazole (100 %), followed by Propiconazole (94.07 %) and Penconazole (94.75 %); Among non-systemic and combi- fungicides, significantly highest average mycelial growth inhibition was observed in Carbendazim 12 WP + Mancozeb 63 WP (85.80 %), followed by Mancozeb (82.59 %) and Copper-oxychloride (76.65 %). Among bioagents tested *T. viride* recorded significantly highest mycelial growth inhibition (87.04 %) of the test pathogen, followed by *T. harzianum* (82.59 %) and *T. koningii* (78.89 %). Among botanicals *A. sativum* was found most fungistatic and recorded significantly highest average mycelial growth inhibition 88.33 %, followed by *A. indica* (78.58 %), *O. sanctum* (73.83 %) and *E. globulus* (66.05 %).

Keyword : *Alternaria carthami*, safflower, fungicides, bioagents, botanicals, mycelial inhibition.

Safflower (*Carthamus tinctorious*) is one of the important oilseed crops of the world valued for its highly nutritious edible oil. Safflower seed contains 25-32 % oil and rich in polyunsaturated fatty acids (Linoleic acid, 78%). Its belongs to family Compositae and believes to be native Afganistan. Safflower is known to suffer from many fungal diseases at different stages of crop growth (Bhale *et al*., 1998). Leaf spot/blight (*Alternaria carthami*), Wilt (*Fusarium oxysporum F.* Sp. *carthami*), Root rot (*Rhizoctinia bataticola*), Powdery mildew (*Erysiphe cicnoralearum* DC) and Anthracnose (*Colletotrichum capsici*). Among these diseases, under present situation leaf spot/ blight caused by *Alternaria carthami* (Chowdhury) has become one of the major constraints in the production and productivity of safflower in the country in general as well as in the state of Maharashtra particularly. The disease (*A. carthami*) has been reported to cause 25 to 60 per cent yield losses in safflower (Singh and Prasad, 2005). The disease has been also reported to reduce drastically the seed size, seed volume test as well as per cent oil content.

Typical symptoms of the disease (*A. carthami*) are appeared as irregular necrotic lesions on leaves and stem. Dark necrotic lesions 2-5 mm in diameter are firstly found on hypocotyls and cotyledons. In mature plant, small brown to dark brown concentric spot of 1-2 mm appear on leaves and brown discoloration appear on the stem, dark brown spot with concentric ring up to 1 cm in diameter appear on leaves which later developed in to large lesion (Mortensen, 1993).

* To whom all correspondence should be addressed.

Safflower cultivars / varieties presently under cultivation do not posses proven field resistance or tolerance and majority of them are more or less prone to the leaf spot / blight disease (*A. carthami*), under such circumstances fungicides provide the most reliable means of controlling foliage diseases. Present day public perceptions and environmental hazards are compelling to search for alternative eco-friendly disease management strategies, for which integration of various cultural, biological and chemical methods might be the solution (Barnwal *et al*., 2011; Jagan *et al*., 2013). Recently, biological control using bioagents and phytoextracts has received much attention in both conventional and organic farming to suppress plant diseases and to overcome some extent the public concerns regarding chemical fungicides (Gardener and Fravel, 2002; Samnels, 2006). Several plant extracts have been demonstrated to possess excellent antifungal properties and their exploitation as bio-fungicides has been emphasized within the broad strategy of environmental protection (Singh *et al*., 2013; Devi *et al*., 2014). An understanding of the role of environmental factors and their consequences on infection, development and spread of the pathogen / diseases is needed to develop sustainable disease management practices (Bal and Kumar, 2014; Singh *et al.* 2014).

MATERIALS AND METHODS

In vitro evaluation of fungicides

Efficacy of six systemic fungicides and seven non-systemic / combi fungicides was evaluated *in vitro* at 500, 1000, 1500 ppm (Systemic fungicides) and 1000, 2000 and 2500 ppm (Non Systemic) concentration against *A. carthami,* applying Poisoned food technique (Nene and Thapliyal, 1993) and using Potato dextrose agar (PDA) as basal culture medium. Based on active ingredient, requisite quantity of the test fungicides was calculated, mixed separately thoroughly with autoclaved and cooled (40 ^{0}C) PDA medium in conical flasks to obtain desired concentrations. This PDA medium amended separately with the test fungicides was then poured (20 ml / plate) aseptically in Petri plates (90 mm dia.) and allowed to solidify at room temperature. For each of the test fungicide and its desired concentrations, three plates / treatment / replication were maintained. After solidification of the PDA medium, all the plates were inoculated aseptically by placing in the centre a 5 mm culture disc obtained from actively growing 7 days old pure culture of *A. carthami* and incubated in an inverted position at 28±2 ^{0}C. Petri plates filled with plain PDA (without any fungicide) and inoculated with the pure culture disc of *A. carthami* were maintained as untreated control.

Observations on radial mycelial growth / colony diameter were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test pathogen. Per cent inhibition of the test pathogen with the test fungicides over untreated control was calculated by applying following formula (Vincent, 1927).

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

Where,

C = growth of the test fungus in untreated control plate

T = growth of the test fungus in treated plate

In vitro evaluation of bioagents

Seven fungal and two bacterial bioagents 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 pathogen (*A. carthami*) grown on PDA were used for the study. Two 5 mm culture discs, one each of the test pathogen and test bioagent were cut out with sterilized cork borer and placed at equidistance, exactly opposite to each other on autoclaved and solidified PDA medium in Petri plates and three plates were incubated at 28±2 ^{0}C. PDA plates inoculated alone with pure culture disc (5 mm) of the test pathogen were maintained as untreated control.

Observations on linear mycelial growth of the test pathogen and test bioagent were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test pathogen. Per cent inhibition of the test pathogen with the test bioagent, over untreated control was calculated by applying following formula (Arora and Upaddhyay, 1978).

$$\text{Per cent Growth Inhibition} = \frac{\text{Colony growth in control plate} - \text{Colony growth in Intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

***In vitro* evaluation of plant extracts / botanicals**

Aqueous extracts of 14 botanicals (as detailed under treatments) were evaluated *in vitro* against *A. carthami*. Leaf / bulb / rhizome extract of the test botanicals were prepared by grinding with mixture-cum grinder. Washed 100 g each leaves / Turmeric rhizome / Onion bulb / Garlic cloves were macerated separately in 100 ml distilled water (w/v) and the macerates obtained were filtered separately 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 obtained formed the standard plant extracts of 100 per cent concentration. These were evaluated (each @ 10 %, 15 % and 20 %) *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 test aqueous extract (100 %) was separately mixed thoroughly with autoclaved and cooled (40 ^{0}C) PDA medium in conical flasks (250 ml cap.) to obtain desired concentrations of 10, 15 and 20 per cent. The PDA medium amended separately with the test aqueous extract was then poured (20 ml / plate) into sterile glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. For each test botanical extract and their respective concentrations, three plates / treatment / replication were maintained and all the treatments were replicated thrice. Upon solidification of the amended PDA medium, all the treatment 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 without any botanical extract and inoculated with mycelial disc of the test pathogen served as untreated control. All these plates were then incubated at 28± 2 ^{0}C temperature for a week or till the untreated control plates were fully covered with mycelial growth of the test pathogen.

Observations on radial mycelial growth / colony diameter of the test pathogen were recorded treatment-wise at 24 hours interval and continued till mycelial growth of the test pathogen 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

Disease management strategies

***In vitro* evaluation of systemic fungicides**

Mycelial inhibition

Results (Table 1 and Plate 1) revealed that all the systemic fungicides tested (each @ 500, 1000 and 1500 ppm) significantly inhibited mycelial growth of *A. carthami*, over untreated control. Further, per cent mycelial inhibition was increased with increase in concentrations of the fungicides tested represented in Fig. 1.

Table 1. *In vitro* bioefficacy of systemic fungicides against *A. carthami*

Tr. No.	Treatments	% Inhibition*at ppm 500	1000	1500	Av.(%)
T_1	Carbendazim50 WP	38.52(22.66)	49.26(29.51)	53.70(32.48)	47.16(28.22)
T_2	Propiconazole 25 EC	94.22(70.44)	100(89.99)	100(89.99)	98.07(83.47)
T_3	Hexaconazole 5 EC	100(89.99)	100(89.99)	100(89.99)	100(89.99)
T_4	Difenconazole 25 EC	83.70(56.87)	89.08(63.00)	93.33(68.95)	88.70(62.94)
T_5	Penconazole 10 EC	90.19(64.40)	94.07(70.17)	100(89.99)	94.75(74.85)
T_6	Thiophanate methyl 70WP	27.41(15.91)	41.48(24.50)	44.63(26.50)	37.84(22.30)
T_{15}	Control(Untreated)	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)
—	S.E. +	0.69	0.45	0.32	—
—	C.D. (P = 0.01)	2.08	1.37	0.97	—

*: Mean of three replications, Dia: Diameter, Av.: Average
Figures in parentheses are arcsine transformed values

Average mycelial growth inhibition recorded with the test systemic fungicides was ranged from 37.84 (Thiophanate methyl) to 100 (Hexaconazole) per cent. However, it was cent per cent with Hexaconazole (100 %), followed by Propiconazole (94.07 %) and Penconazole (94.75 %) and Difenconazole (88.70%). Whereas, it was comparatively minimum with Thiophanate methyl (37.84 %) and Carbendazim (47.16 %).

These results are in conformity with the earlier findings of those workers who reported systemic fungicides *viz*., Hexaconazole, Propiconazole, Penconazole, Difenconazole, Thiophanate methyl and Carbendazim at various concentrations had significantly inhibited mycelial growth of *A. carthami* infecting safflower (Murumkar *et al*., 2009; Taware *et al*., 2014), *A. helianthi* infecting sunflower (Amaresh and Nargund, 2004), *A. alternata* infecting sesame (Bavaji *et al.*, 2012).

***In vitro* evaluation of non-systemic and combi-fungicides**

Mycelial inhibition

Results (Table 2, Plate 2 and Fig.

Table 2. *In vitro* bioefficacy of non-systemic and combi- fungicides against *A. carthami*

Tr. No.	Treatments	% Inhibition*at ppm 1000	2000	2500	Av.(%)
T_7	Chlorotholonil	46.30(27.82)	50.74(30.49)	52.96(31.98)	50.00(30.10)
T_8	Ridomil MZ72 WP	51.67(31.23)	53.89(32.60)	56.11(34.13)	53.89(32.65)
T_9	Carbendazim 12 WP + Mancozeb 63 WP	83.15(56.62)	84.81(58.03)	89.44(63.48)	85.80(59.38)
T_{10}	Copper-oxychloride 50WP	71.67(46.23)	77.74(50.72)	80.54(53.64)	76.65(50.20)
T_{11}	Copper-hydroxide77 WP	66.30(41.24)	70.74(45.03)	71.67(45.77)	69.57(44.01)
T_{12}	Mancozeb75 WP	80.00(53.84)	81.85(54.95)	85.93(59.24)	82.59(56.01)
T_{13}	Propineb70 WP	57.22(34.90)	61.48(37.93)	63.52(39.43)	60.74(37.42)
T_{14}	Curzate M872 WP	64.62(40.54)	66.48(41.67)	68.52(43.25)	66.54(41.82)
T_{15}	Control(Untreated)	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)
—	S.E. +	0.46	0.65	0.63	—
—	C.D. (P = 0.01)	1.37	1.94	1.87	—

*: Mean of three replications, Dia: Diameter, Av.: Average
Figures in parentheses are arcsine transformed values

Table 3. *In vitro* bioefficacy of bioagents against *A. carthami*

Tr. No.	Treatments	Colony Dia. of test pathogen * (mm)	% Inhibition
Fungal antagonists			
T_1	*Trichoderma viride*	11.67	87.04(60.54)
T_2	*T. harzianum*	15.67	82.59(55.70)
T_3	*T. hamatum*	23.33	74.07(47.80)
T_4	*T. koningii*	19.00	78.89(52.08)
T_5	*T. longibrachiatum*	27.33	69.63(44.13)
T_6	*T. (Gliocladium) virens*	31.67	64.81(40.41)
T_7	*Aspergillus niger*	39.83	55.74(36.60)
Bacterial antagonists			
T_8	*Bacillus subtilis*	36.33	59.63(29.63)
T_9	*Pseudomonas fluorescens*	45.05	49.33(33.87)
T_{10}	Control (Untreated)	90.00	00.00(00.00)
—	S.E. +	0.78	0.75
—	C.D. (P = 0.01)	2.30	2.22

* : Mean of three replications, Dia.: Diameter,
Figures in parentheses are arcsine transformed values

2) revealed that all non-systemic and combi-fungicides tested (each @ 1000, 2000 and 2500 ppm) significantly inhibited mycelial growth of *A. carthami*, over untreated control. Further, per cent mycelial inhibition was increased with increase in concentrations of the fungicides tested.

Average mycelial growth inhibition recorded with the test non-systemic and combi-fungicides was ranged from 50.00 (Chlorotholonil) to 85.80 (Carbendazim 12 WP + Mancozeb 63 WP) per cent. However, it was highest average mycelial growth inhibition with Carbendazim 12 WP + Mancozeb 63 WP (85.80 %), followed by Mancozeb (82.59 %), Copper-oxychloride (76.65 %), Copper-hydroxide (69.57 %), Curzate (66.54 %), Propineb (60.74 %), Ridomil MZ (53.89 %) and Chlorotholonil (50.00 %).

Thus, six systemic, six non-systemic and two combi- fungicides tested were found fungistatic against *A. carthami*. However, on the basis of order of merit systemic fungicides *viz*., Hexaconazole, Propiconazole, Penconazole and Difenconazole and non-systemic / combi fungicides Carbendazim 12 WP + Mancozeb 63 WP (SAAF 75 WP),

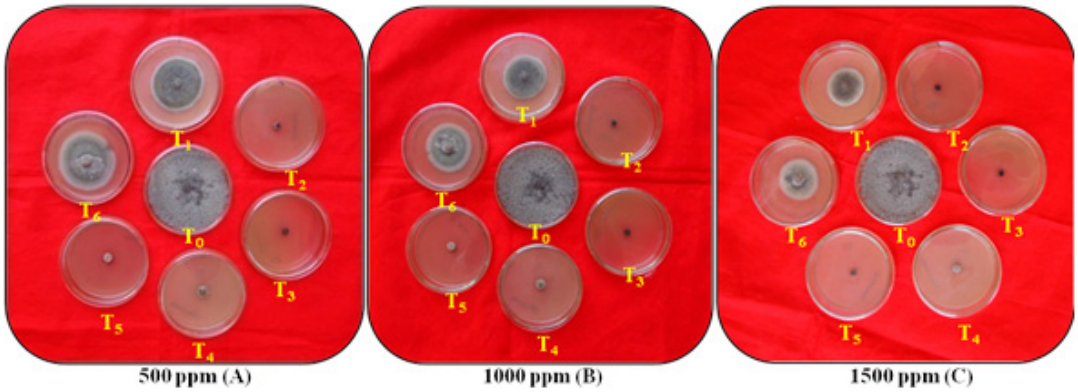

Plate 1. *In vitro* effect of systemic fungicides at various concentrations on growth and inhibition of *A. carthami*

Table 4. *In vitro* bioefficacy of botanicals against *A. carthami*

Tr. No.	Treatments	Av. (mm)	% Inhibition 10	15	20	Av. (%)
T_1	Onion(*Allium cepa*)	44.22	50.37(23.12)	55.56(33.75)	57.78(35.30)	50.87(30.72)
T_2	Ghaneri(*Lantana camera*)	44.28	46.30(27.58)	50.37(30.24)	55.74(33.87)	50.80(30.56)
T_3	Tulsi(*Oscimum sanctum*)	23.56	65.74(41.10)	76.49(49.89)	79.26(52.43)	73.83(47.81)
T_4	Gliricidia(*Gliricidia sepium*)	77.78	07.04(40.37)	14.07(80.87)	19.63(11.32)	13.58(44.19)
T_5	Neem(*Azardirachta indica*)	19.28	72.96(46.86)	79.26(52.43)	83.52(56.64)	78.58(51.98)
T_6	Garlic(*Allium sativum*)	10.50	83.70(56.87)	88.52(62.27)	92.78(68.15)	88.33(62.43)
T_7	Bouganveillia(*B. spectabilis*)	72.06	14.44(83.04)	19.44(11.21)	25.93(15.03)	19.94(36.43)
T_8	Datura(*Datura metal*)	41.33	51.48(30.98)	56.67(34.52)	61.30(37.80)	56.48(34.43)
T_9	Eucalyptus(*Eucalyptus globulus*)	30.56	59.63(36.60)	65.93(41.24)	72.59(46.55)	66.05(41.46)
T_{10}	Periwinkle(*Vinca rosea*)	51.17	35.93(21.05)	47.96(28.66)	50.37(30.24)	44.75(26.65)
T_{11}	Turmeric(*Curcuma longa*)	32.33	53.89(32.61)	60.74(37.40)	67.41(42.39)	60.68(37.47)
T_{12}	Custard apple(*Annona squimosa*)	64.78	21.48(12.41)	27.04(15.69)	35.56(20.83)	28.03(16.31)
T_{13}	Parthenium(*P. hysterophorus*)	59.11	25.74(14.91)	35.37(20.71)	41.85(24.74)	34.32(20.12)
T_{14}	Karanj(*Pongamia pinnata*)	53.44	32.96(19.24)	41.85(24.74)	47.04(28.06)	40.62(24.01)
T_{15}	Control (Untreated	90.00	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)
—	S.E. +	—	0.64	0.41	0.67	—
—	C.D. (P = 0.01)	—	1.86	1.18	1.94	—

* : Mean of three replications, Dia.: Diameter, Av.: Average, Conc.: Concentration,
Figures in parentheses are arcsine transformed values

Mancozeb, Copper-oxychloride, Copper-hydroxide and Curzate etc were found most promising against *A. carthami*, causing leaf spot / blight in safflower.

Non-systemic fungicides *viz*., Mancozeb, Copper-oxychloride, Copper hydroxides, Propineb and Chlorothalonil and Combi- fungicides (systemic+contact) *viz*., Carbendazim+Mancozeb, Metalaxyl+Mancozeb were also reported to cause cent-per cent or significantly maximum mycelial growth inhibition of *Alternaria* leaf spot / blight causing fungal pathogens such as *A. carthami* infecting safflower (Murumkar *et al*., 2009; Taware *et al*., 2014), *A. helianthi* infecting sunflower (Mathivanan and Prabhavathy, 2007; Mesta *et al*., 2009).

***In vitro* evaluation of bioagents**

The results obtained on mycelial growth and inhibition of *A. carthami* with seven fungal and two bacterial antagonists are presented in Table 3, Plate 3 and Fig. 3.

Results (Table 3) revealed that all the bioagents evaluated exhibited fungistatic / antifungal activity against *A. carthami* and significantly inhibited its growth, over untreated control. Of the antagonists tested, *T. viride* was found most effective with highest mycelial

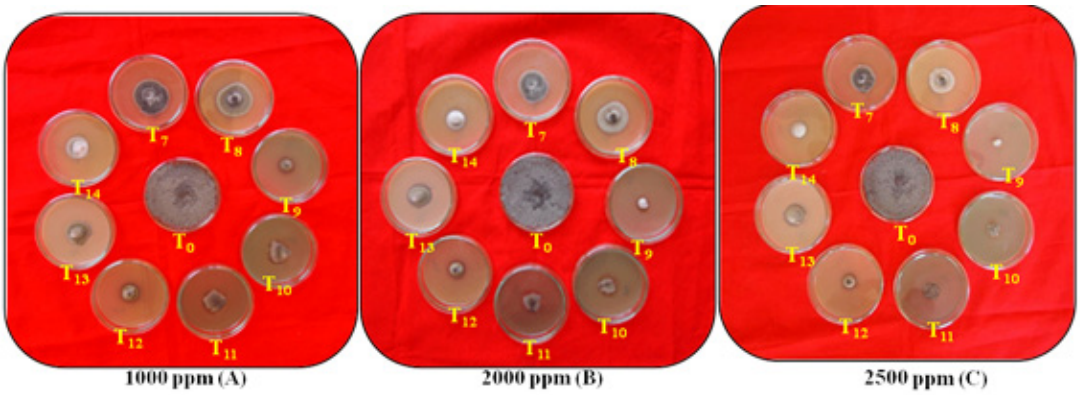

Plate 2. *In vitro* effect of non-systemic and combi- fungicides at various concentrations on growth and inhibition of *A. carthami*

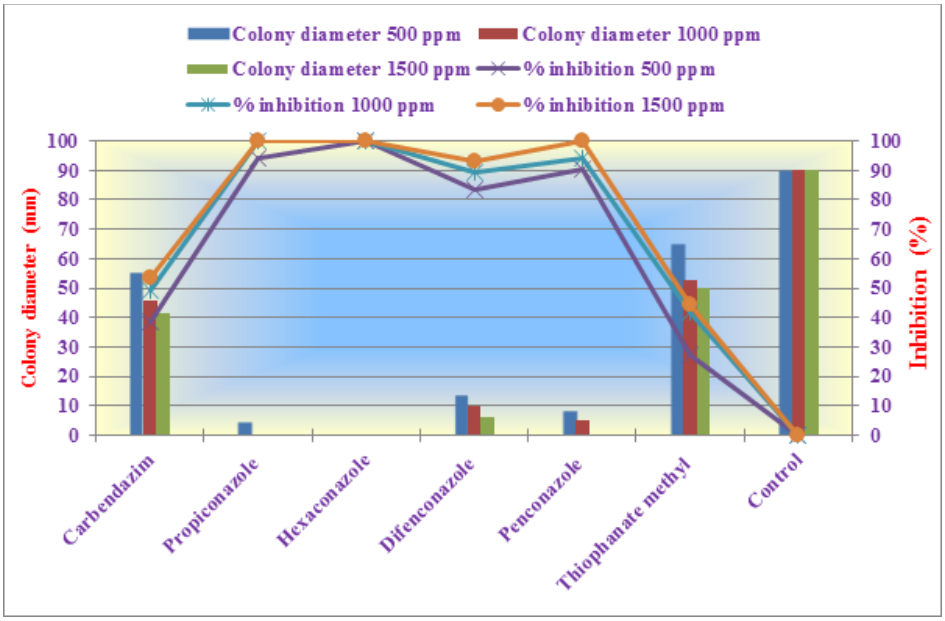

Fig. 1. *In vitro* bioefficacy of systemic fungicides against *A. carthami*

growth inhibition (87.04 %) of the test pathogen. The second and third most inhibitory antagonists found were *T. harzianum* and *T. koningii* with and inhibition of 82.59 and 78.89 per cent, respectively. There were followed by *T. hamatum* (74.07 %), *T. longibrachiatum* (69.63 %), *T. virens* (64.81%), *Bacillus subtilis* (59.63 %), *A. niger* (55.74 %) and *P. fluorescens* (49.33 %). Thus, the bioagents *viz*., *T. viride*, *T. harzianum*, *T. koningii*, *T. hamatum* were found most potential antagonists against *A. carthami* (Fig. 3).

These results are in conformity with the earlier findings of those workers who reported bioagents *viz*., *T. viride, T. harzianum, T. koningii* and *T. hamatum* had significantly inhibited mycelial growth of *A. carthami* infecting safflower (Taware *et al.*, 2014), *A. brassicae* infecting rapeseed-mustard (Reshu and Khan, 2012), *A. alternata* / *A. sesame* infecting sesame (Akbari and Parakhia, 2007; Savitha *et al.,* 2011).

Plate 3. *In vitro* effect of bioagents on growth and inhibition of *A. carthami*

The fungistatic / antifungal action exerted by the species of *Trichoderma* and *A. niger* against *A. carthami* and other species of *Alternaria* may be attributed to their production of volatile and non-volatile substances, cell wall degrading enzymes (glucanases, B1, 3 glucanase), the phenomenon of competition, lysis and antibiosis.

***In vitro* evaluation of plant extracts / botanicals**

Results (Table 4) revealed that all the 14 botanicals tested (each @ 10, 15 and 20 %) exhibited a wide range of radial mycelial growth of *A. carthami* (Plate 4 and Fig. 4) and it was decreased drastically with increase in concentrations of the test botanicals from 10 to 20 per cent.

Mycelial inhibition

Average mycelial growth inhibition recorded with the test botanicals was ranged from 13.58 (*G. maculata*) to 88.33 (*A. sativum*) per cent. However, it was highest with *A. sativum* (88.33 %), followed by *A. indica* (78.58 %), *O. sanctum* (73.83 %), *E. globulus* (66.05 %), *C. longa* (60.68 %), *D. metal* (56.48 %), *Allium cepa* (50.87 %) and *L. camera* (50.80 %). Rest of the test botanicals recorded average mycelial growth inhibition in the

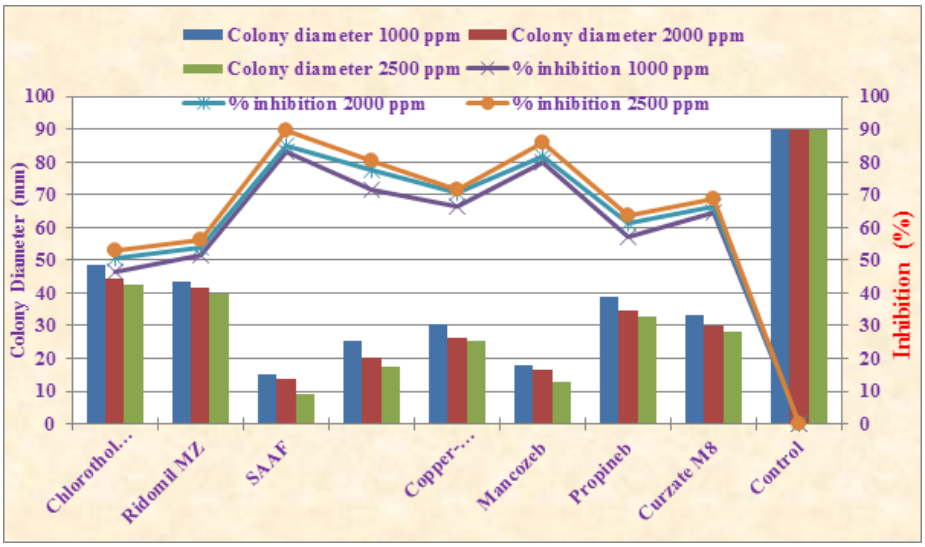

Fig. 2. *In vitro* bioefficacy of non-systemic and combi- fungicides against *A. carthami*

range of 28.03 to 44.75 per cent; whereas, it was significantly least with *G. sepium* (13.58 %) and *B. spectabilis* (19.94 %).

Thus, on the basis of antifungal activity, the botanicals found most effective in the order of merit were *A. sativum, A. indica, O. sanctum, E. globules, C. longa, D. metal, Allium cepa, L. camera, V. rosea* and *P. pinnata*, which after their further confirmation could be exploited for control of leaf spot / blight of safflower (*A. carthami*).

These results are in conformity with the earlier findings of those workers who reported plant extracts / botanicals *viz*., *A. sativum, A. indica, O. sanctum, E. globules, C. longa,* at various concentrations had significantly inhibited mycelial growth of *A. carthami* infecting safflower (Ranaware *et al*., 2010; Usha *et al*., 2012; Taware *et al.*, 2014).

In the present study, of the 14 phytoextracts *A. sativum* extract was found most effective which caused substantial inhibition (> 88%) of *A. carthami.* The antifungal activity of *A. sativum* has been attributed to the presence of diallyl sulphide and other compounds like alllisatin I, II and garli phytocide (Cavallito *et al.,* 1974; Sharma and Prasad, 1980).

A number of phytoextracts / botanicals have been studied and reported with potential inhibitory action against many phytopathogenic fungi, bacteria and viruses. The presence of various secondary metabolites *viz.,* alkaloids, quaternary alkaloids, cumarins, flavanoids, steroids / terpenoids, phenolics etc with potential

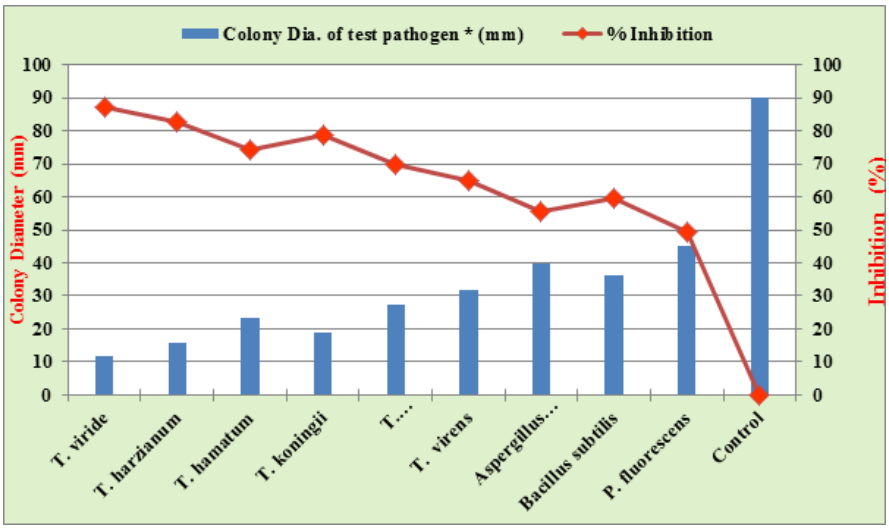

Fig. 3. *In vitro* bioefficacy of bioagents against *A. carthami*

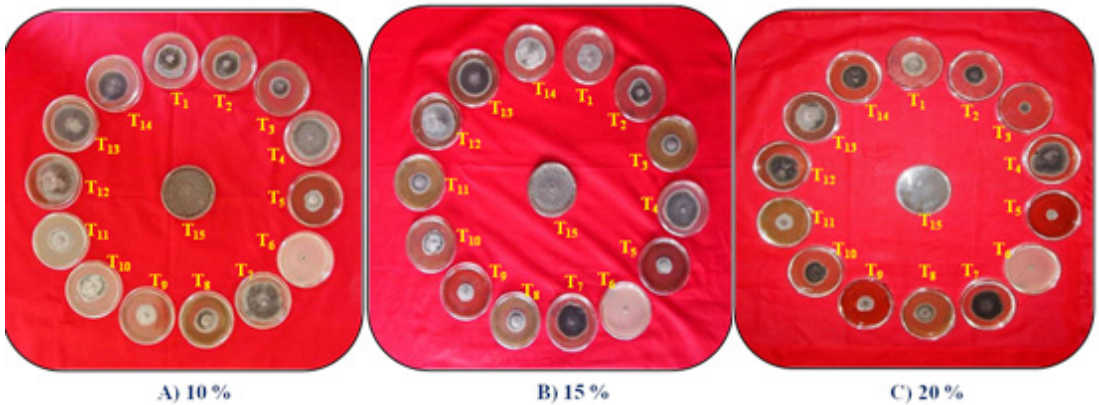

Plate 4. *In vitro* bioefficacy of botanicals against *A. carthami*

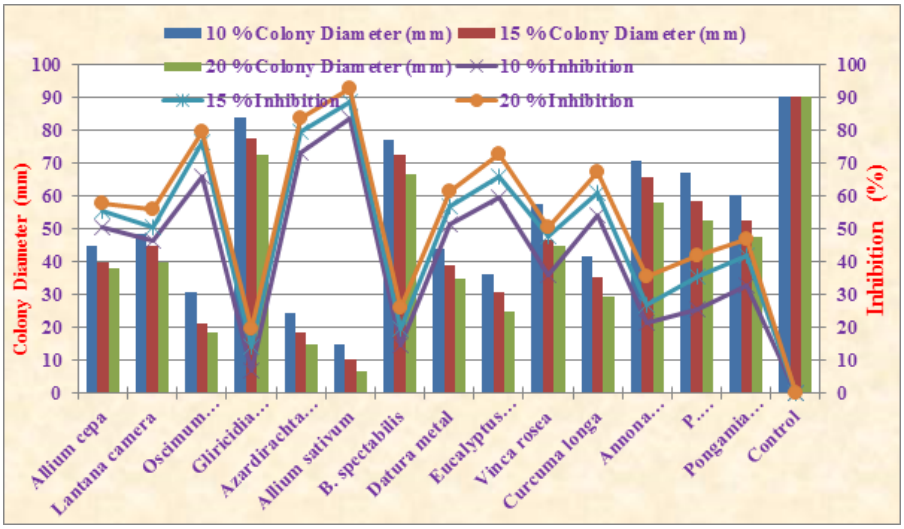

Fig. 4. *In vitro* bioefficacy of botanicals against *A. carthami*

antifungal activity were reported in various plant extracts (Alice,1984; Aswal, *et al.*,1984; Abraham *et al.,*1986; Chopra *et al.,* 1992).

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