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To evaluate *in vitro* the efficacy of fungicides, bio-control agents and plant extract against *Colletotrichum capsici*

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Abstract
Colletotrichum capsici infection will be higher in the mature stage of chilli plant than in the early stage of plant (Krairuan *et al*., 2008). The fungus prefers warm humid environment for spreading the anthracnose disease uniformly and effectively. All the two contact and six systemic fungicides evaluated *in vitro* were found fungistatic against *Colletotrichum capsici.* Among all these fungicides, Tricyclazole was found to be most effective with significantly maximum mycelial growth inhibition and least mycelial growth followed by fungicides *viz.* Azostrobin, Tebuconazole, Difenconazole, Propiconazole, Carbendazim + Mancozeb. While comparatively minimum average radial mycelial growth inhibition was recorded with Hexaconazole followed by Propineb. All the seven fungal and one bacterial antagonists evaluated *in vitro* were found antifungal against *Colletotrichum capsici* exhibited significant mycelial growth inhibition. However, *Trichoderma asperallum*, *Pseudomonas fluorescens*, *Trichoderma harzianum and Gliocladium virens* recorded significantly highest mycelial growth inhibition. Rest of the bioagents, *Trichoderma ligronum*, *Trichoderma koningii and Trichoderma longibrachiatum* and *Trichoderma hamatum* tested also caused significant mycelial inhibition of the test pathogen.

Keywords: *Colletotrichum capsici*, efficacy of fungicides, *in vitro*

Introduction
Chilli crop is affected by several fungal, bacterial and viral diseases: (Anand *et al.*, 2009). Among the major fungal diseases; Damping off (*Pythium aphanidermatum*), Powdery mildew (*Leveillula taurica*), Anthracnose or fruit rot (*Colletotrichum capsici*) and *Cercospora* leaf spot (*Cercospora capsici*) are the major diseases. The important bacterial diseases includes; Bacterial wilt (*Ralstonia solanacearum*), Leaf spot (*Xanthomonas vesicatoria*), nematode diseases like Root knot and Root gall caused by *Meloidogyne spp.* and viral diseases like Chilli mosaic, leaf roll and leaf curl. Among the large number of diseases affecting chilli cultivation, anthracnose disease caused by *Colletotrichum species*, bacterial wilt by *Psuedomonas solanacearum* and viral diseases like chilli mosaic virus (CMV) infection have been most detrimental to chilli production.

Anthracnose of chilli was first reported from New Jersey, USA, described the causal agents as *Gloeopsorium piperatum* and *Colletotrichum nigrum.* These taxa were then considered as synonyms of *Colletotrichum gloeosporiodes* heavy crop losses worldwide. Specifically, *Colletotrichum* is an asexual genus belonging to phylum Ascomycete and Coeleomycetes class of Fungi imperfectii. The disease has been reported from many countries including India, United States of America, Nigeria, Bangladesh and Indonesia. Anthracnose disease is a major problem in India and first reported in India from Coimbatore of Madras Presidency. These disease caused by more than one Colletotrichum species including; Colletotrichum acutatum, Colletotrichum capsici, Colletotrichum gloeosporioides and Colletotrichum coccodes. Colletotrichum capsici infection will be higher in the mature stage of chilli plant than in the early stage of plant. The fungus prefers warm humid environment for spreading the anthracnose disease uniformly and effectively. The fungus is primarily invades into injured or weakened tissues of plants, produces various specialized structures during infection process.

In tropical countries, high moisture condition due to monsoon rain during June-October favors sporulation of Colletotrichum capsici which enhances the fruit rot disease incidence and helps outbreak of the disease. Hence, the objective of present study was to evaluate *in vitro* the efficacy of fungicides, bio-control agents and plant extract against *Colletotrichum capsici*.

Material and Methods

In vitro evaluation of fungicides

Experimental details

Design: C.R.D.
Replications: Three
Treatments: Nine

Table 1: Treatment details

Sr. No.	Common Name	Trade Name	Concentration
T_1	Carbendazim + Mancozeb	Saaf 75 WP	0.05%, 0.1%0.2%
T_2	Propineb	Antracol 70 WP	0.05%, 0.1% 0.2%
T_3	Hexaconazole	Contaf 5% EC	0.025%, 0.05% 0.1%
T_4	Propiconazole	Tilt 25 EC	0.025%, 0.05% 0.1%
T_5	Difenconazole	Score 25 EC	0.025%, 0.05% 0.1%
T_6	Tebuconazole	Folicur 25 EC	0.025%, 0.05% 0.1%
T_7	Azoxystrobin	Amistar 25 EC	0.025%, 0.05% 0.1%
T_8	Tricyclazole	Beam 75%WP	0.025%, 0.05% 0.1%
T_9	Control		

Observations on radial mycelial growth of the test fungus were recorded at 24 hrs interval and continued till growth of the test pathogen in untreated control plate was fully covered. Per cent inhibition of the test pathogen was calculated by applying the formula.

$$\text{Per cent inhibition (R)} = \frac{C - T}{C} 100$$

Where,
C= Growth of the test fungus in untreated control plates. T= Growth of the test fungus in treated plates.

In vitro evaluation of bioagents

Experimental Details : Design
: C.R.D.
Replications : Three
Treatments : Nine

Table 2: Treatment details

Tr. No.	Treatments
T_1	*Trichoderma asperallum*
T_2	*Trichoderma harzianum*
T_3	*Trichoderma hamatum*
T_4	*Pseudomonas fluorescens*
T_5	*Trichoderma ligronum*
T_6	*Trichoderma koningii*
T_7	*Trichoderma longibrachiatum*
T_8	*Gliocladium virens*
T_9	*Control*

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

Where,

$$\text{Per cent inhibition (R)} = \frac{C - T}{C} 100$$

C= Growth of the test fungus in untreated control plates.
T= Growth of the test fungus in treated plates.

In vitro evaluation of botanicals (plant extracts)

Experimental details

Design : C.R.D.
Replication : Three
Treatment : Eight
T_1 : karanj (Leaves extract)
T_2 : Onion (Bulbextract)
T_3 : Garlic (Bulbextract)
T_4 : Turmeric (Rhizome extract)
T_5 : Neem (Leaves extract)
T_6 : Ginger (Rhizome extract
T_7 : Tulsi (Leaves extract)
T_8 : Control (Untreated).

Observations on radial mycelial growth of the test fungus were recorded at 24 hrs interval and continued till growth of the test pathogen in untreated control plate was fully covered. Per cent inhibition of the test pathogen was calculated by applying the formula.

$$\text{Per cent inhibition (R)} = \frac{C - T}{C} 100$$

Where,
C= Growth of the test fungus in untreated control plates.
T= Growth of the test fungus in treated plates.

Results and Discussion

In vitro efficacy of fungicides

A total 8 fungicide evaluated *in vitro* against *Colletotrichum capsici* exhibited a wide range of mycelial growth and inhibition of the test pathogen. The results obtained are presented in the Table-1 and depicted in Fig.- 3 and PLATE-5 (A).

Radial mycelial growth

Results (Table 1, Fig 1, Plate 5 B) indicated that the mycelial growth of *Colletotrichum capsici* was significantly differed with all the contact fungicides evaluated at 0.05%, 0.1% and 0.2% and systemic fungicides 0.025%, 0.05% and 0.1%. (Table 1, Fig 3, Plate 5 B).

Systemic fungicides at 0.25% and contact fungicides at 0.05%, radial mycelial growth of the test pathogen were ranged from 20.00 mm (Tricyclazole) to 53.33 mm (Propineb). However, significantly least mycelial growth was recorded with the fungicide Tricyclazole (20.00 mm) followed by the fungicides *viz* Azoxystrobin (24.00 mm), Difenconazole (26.33 mm), Tebuconazole (27.66 mm), Propiconazole (31.66 mm), Carbendazim + Mancozeb (36.00 mm) and Hexaconazole (39.66 mm) which were at par with

each other. Whereas, comparatively maximum radial mycelial growth was recorded with Propineb (53.33 mm).

Systemic fungicides at 0.05% and contact fungicides at 0.1%, radial mycelial growth of the test pathogen were ranged from 18.66 mm (Tricyclazole) to 36.33 mm (Propineb). However, significantly least mycelial growth was recorded with the fungicide Tricyclazole (18.66 mm) followed by the fungicides *viz* Tebuconazole (21.00 mm), Azoxystrobin (22.66 mm), Difenconazole (25.00 mm), Propiconazole (29.33 mm), Carbendazim + Mancozeb (33.00 mm) which were at par with each other. Whereas, comparatively maximum radial mycelial growth was recorded with Propineb (36.33 mm).

Systemic fungicides at 0.1% and contact fungicides at 0.2%, radial mycelial growth of the test pathogen were ranged from 9.66 mm (Azostrobin) to 31.00 mm (Propineb and Hexaconazole). However, significantly least mycelial growth was recorded with the fungicide Azoxystrobin (9.66 mm) followed by the fungicides *viz* Tebuconazole (12.66 mm), Tricyclazole (15.00 mm) Propiconazole (18.00 mm), Difenconazole (22.00 mm) and Carbendazim + Mancozeb (23.33 mm) which were at par with each other. Whereas, comparatively maximum radial mycelial growth was recorded with Propineb and Hexaconazole (31.00 mm). Pathogen. The results obtained are presented in the Table-3 and depicted in Fig. and PLATE.

Table 3: *In vitro* efficacy of fungicides against mycelial growth and inhibition of *Colletotrichum capsici*

Tr. No.	Treatments	Colony Dia. *(mm) at Percentage			Av. (mm)	% Inhibition* at Percentage			Av. inhibition (%)
		0.05	0.1	0.2		0.05	0.1	0.2	
T_1	Carbendazim + Mancozeb	36.00 (36.86)	33.00 (35.06)	23.33 (28.88)	30.77 (33.69)	60.00 (50.76)	63.3 (57.73)	74.07 (59.38)	65.80 (54.21)
T_2	Propineb	53.33 (46.90)	36.33 (37.06)	31.00 (33.83)	40.22 (39.36)	40.74 (39.66)	59.63 (50.55)	65.55 (54.05)	55.30 (48.04)
		0.025	0.05	0.1		0.025	0.05	0.1	
T_3	Hexaconazole	39.66 (39.03)	33.33 (35.26)	31.00 (33.83)	34.66 (36.06)	55.93 (48.40)	62.96 (52.51)	65.55 (54.05)	61.48 (51.63)
T_4	Propiconazole	31.66 (34.24)	29.33 (32.79)	18.00 (25.10)	26.33 (30.87)	64.82 (53.62)	67.41 (55.18)	80.00 (63.43)	70.74 (57.25)
T_5	Difenconazole	26.33 (30.87)	25.00 (30.00)	22.00 (27.97)	24.44 (29.62)	70.74 (57.25)	72.22 (58.19)	75.55 (66.36)	72.83 (58.58)
T_6	Tebuconazole	27.66 (31.73)	21.00 (27.27)	12.66 (20.84)	20.44 (26.87)	69.26 (56.32)	76.66 (61.11)	85.93 (67.96)	77.28 (61.53)
T_7	Azoxystrobin	24.00 (29.33)	22.66 (28.42)	9.66 (18.10)	18.77 (25.67)	73.33 (38.90)	74.82 (59.88)	89.26 (70.86)	79.13 (62.81)
T_8	Tricyclazole	20.00 (26.54)	18.66 (25.59)	15.00 (22.78)	17.88 (25.01)	77.77 (61.86)	79.26 (76.26)	83.33 (65.90)	80.12 (63.52)
T_9	Control	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)	00.00	00.00	00.00	00.00
	S.E.+	5.79	4.06	0.82		0.63	0.54	0.72	--
	C.D.(P=0.01)	1.93	1.35	2.46		1.91	1.63	2.15	--

*: Mean of three replications, Dia: Diameter. Figures in parenthesis are arc sine transformed value

Plate

(A) 0.1%.

T1: Carbendazim 25%+Mancozeb 50%
T2: Propineb25 EC
T3: Hexaconzole
T4: Propiconazole
T5: Difenconazole
T6: Tebuconazole
T7: Azoxystrobin
T8: Tricyclazole
T9: Control

In vitro* efficacy of fungicides on radial mycelial growth and inhibition of *Colletotrichum capsici

Plate

(A) 0.05%

(B) 0.025%,

T1: Carbendazim 25%+Mancozeb 50%
T2: Propineb25 EC
T3: Hexaconzole
T4: Propiconazole
T5: Difenconazole

T6: Tebuconazole
T7: Azoxystrobin
T8: Tricyclazole
T9: Control

Mycelial growth inhibition

The results (Table and Fig.) indicated that all contact fungicides evaluated at 0.05%, 0.1% and 0.2% and systemic fungicides 0.025%, 0.05% and 0.1% which significantly inhibited mycelial growth of the test fungus over untreated control (00.00%).

Further, it was found that percentage growth inhibition was increased with the increase in concentrations of the fungicides tested (PLATE). Systemic fungicides at 0.025% and contact fungicides at 0.05%, percentage mycelial growth inhibition were ranged from 40.74% (Propineb) to 77.77% (Tricyclazole). However, significantly highest percentage of mycelial growth inhibition was recorded with Tricyclazole 77.77%. This was followed by the fungicides *viz.*, Azoxystrobin (73.33%) Difenconazole (70.74%), Tebuconazole (69.26%), Propiconazole (64.82%), Carbendazim + Mancozeb (60.00%) and Hexaconazole (55.93%). Whereas, comparatively least percentage of mycelial growth inhibition was recorded with Propineb (40.74%). Systemic fungicides at 0.05% and contact fungicides at 0.1 percentage, mycelial growth inhibition was ranged from 79.26% (Tricyclazole) to 59.63% (Propineb). However, significantly highest percentage of mycelial growth inhibition was recorded with Tricyclazole (79.26%) followed by the fungicides *viz* Tebuconazole (76.66%), Azoxystrobin (74.82%), Difenconazole (72.22%), Propiconazole (67.41%), Carbendazim + Mancozeb (63.33%) and Hexaconazole (62.96%). Whereas, comparatively least percentage of mycelial growth inhibition was recorded with Propineb (59.63%).

Systemic fungicides at 0.1% and contact fungicides at 0.2%, mycelial growth inhibition were ranged from 89.26% (Azostrobin) to 65.55% (Propineb and Hexaconazole). However, Significantly highest percentage of mycelial growth inhibition was recorded with Azoxystrobin (89.26%) followed by the fungicides *viz* Tebuconazole (85.93%), Tricyclazole (83.33%), Propiconazole (80.00%), Difenconazole (75.55%) and Carbendazim + Mancozeb (74.07%) Whereas, comparatively least percentage of mycelial growth inhibition was recorded with Propineb and Hexaconazole (65.55%).

The present results are also in agreement with the results obtained by Hegde *et al.,* (2002b) tested the efficacy of three triazole fungicides *viz.*, Hexaconazole (0.1%), Propiconazole (0.1%) and Triadimefon (0.1%) against fruit rot pathogen *Colletotrichum capsici* by poison food technique and reported that significant inhibition of mycelial growth was recorded with all three fungicides.

***In vitro* evaluation of bioagents/ antagonists**

The results obtained on mycelial growth and inhibition *Colletotrichum capsici* with seven fungal and one bacterial antagonists are presented in (Table, Fig. and PLATE).

Table 4: *In vitro* efficacy of different bioagents against mycelial growth and inhibition of *Colletotrichum capsici*

Tr. No.	Treatments	Colony Dia. of test pathogen * (mm)	% Inhibition
T_1	*Trichoderma asperallum*	17.00 (24.35)	81.11 (64.23)
T_2	*Trichoderma harzianum*	26.65 (31.08)	70.38 (57.02)
T_3	*Trichoderma hamatum*	45.00 (42.13)	50.00 (45.00)
T_4	*Pseudomonas fluorescens*	23.00 (28.65)	74.44 (59.63)
T_5	*Trichoderma ligronum*	32.66 (34.85)	63.71 (52.95)
T_6	*Trichoderma koningii*	41.66 (40.19)	53.71 (47.12)
T_7	*Trichoderma longibrachiatum*	43.33 (41.16)	51.85 (46.06)
T_8	*Gliocladium virens*	28.66 (32.36)	68.15 (55.64)
T_9	Control	90.00 (71.56)	00.00
	S.E. +	1.75	0.54
	C.D. (P=0.01)	5.23	1.63

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

The results are presented in Table, Plate and depicted graphically in Fig 4.-revealed that, all the antagonists tested against *Colletotrichum capsici* were effective in checking the growth of the pathogen. Out of eight antagonists tested, least mycelial growth of the test pathogen was recorded in *Trichoderma asperallum* (17.00 mm) which was at par with *Pseudomonas fluorescens* (23.00 mm) followed by *Trichoderma harzianum* (26.65 mm), *Gliocladium virens* (28.66 mm), *Trichoderma ligronum* (32.66 mm), *Trichoderma koningii (41.66 mm) and Trichoderma longibrachiatum* (43.33 mm) while maximum growth recorded with *Trichoderma hamatum* (45.00 mm).

The result presented in Table-4 revealed that, *Trichoderma asperallum* (81.11%), *Pseudomonas fluorescens* (74.44%), *Trichoderma harzianum* (70.38%) *Gliocladium virens* (68.15%), *Trichoderma ligronum* (63.71%) *and Trichoderma koningii* (53.71%) significantly inhibited the pathogen. whereas, *Trichoderma longibrachiatum* (51.85%) and *Trichoderma hamatum* (50.00%) recorded least effective. It is evident from these studies that among all the antagonists evaluated by dual culture method, *Trichoderma asperallum*, *Pseudomonas fluorescens* and *Gliocladium virens* consistently showed strong antagonistic property against *C. capsici* compared to the other antagonists tested hence considered as potential antagonists.

Plate

T_1: *T. asperallum*
T_2: *T. harzianaum*
T_3; *T. hamatum*
T_4: *Pseudomonas fluorescens*
T5: *T. lignorum*
T_6: *T. Koningii*
T_7: *T. longibrachiatum*
T_8: *Gliocladium virens*
T_9: Control

In vitro* efficacy of bioagents on mycelial growth and inhibition of *Colletotrichum

***In vitro* evaluation of plant extracts / botanicals:** In present studies total botanicals were tested for their efficacy against *Colletotrichum capsici* at 15% and 20% concentration by employing poisoned food technique and data are presented in (Table, Fig, and Plate). Results revealed that all the botanicals tested were effective in inhibiting mycelial growth of *Colletotrichum capsici* over untreated control.

Table 5: *In vitro* efficacy of different botanicals/plant extract against mycelial growth and inhibition of *Colletotrichum capsici*

Tr. No.	Treatments	Colony Diameter (mm)*		Ave. (mm)	% Inhibition at Conc.		Ave. (mm)
		@ 15% Concentration	@ 20% Concentration		@15%	@ 20%	
T_1	Karanj	66.00 (54.33)	45.66 (42.51)	55.83 (48.34)	26.66 (31.08)	49.26 (44.57)	37.96 (38.03)
T_2	Onion	36.66 (37.26)	33.00 (35.06)	34.83 (36.16)	59.26 (50.33)	63.33 (52.73)	61.29 (51.52)
T_3	Garlic	47.33 (43.46)	20.00 (26.56)	33.66 (35.46)	47.41 (43.51)	77.77 (61.86)	62.59 (52.29)
T_4	Turmeric	30.00 (33.21)	11.00 (19.36)	20.50 (26.92)	66.66 (54.73)	87.77 (69.53)	77.21 (61.48)
T_5	Neem	21.00 (27.27)	17.00 (24.35)	19.00 (25.84)	76.66 (6.11)	81.11 (64.23)	78.88 (62.64)
T_6	Ginger	41.00 (39.81)	31.33 (34.03)	36.16 (36.96)	54.44 (47.54)	65.18 (53.83)	59.81 (50.65)
T_7	Tulsi	43.00 (40.97)	29.32 (32.78)	36.16 (36.96)	52.22 (46.27)	67.42 (55.19)	59.81 (50.65)
T_8	Control	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)	00.00	00.00	00.00
S.E. +		4.24	4.37		0.54	0.533	
C.D. (P= 0.05)		1.40	1.44		1.65	1.61	

*****: Mean of three replications, Dia: Diameter
Figures in parenthesis are arc sine transformed value

At 15% concentration out of seven botanicals tested the Neem leaf extract was found to be most effective and recorded least mycelial growth (21.00 mm) with highest percent mycelial inhibition (76.66%) of *Colletotrichum capsici* and statistically significant over rest of the botanicals followed by Turmeric rhizome extract which recorded

(A) @ 15%

Plate VIII

(C) @ 20%

In vitro* efficacy of plant extract/botanicals at (A) 15%, (B) 20% on growth and inhibition of *Colletotrichum

Diameter and percent mycelial inhibition of 30.00 mm and (66.66%) and statistically significant over rest of the botanicals. Onion bulb extract recorded colony diameter of 36.66 mm and corresponding inhibition of 59.26% and statistically at par with Ginger extract (41.00 mm and 36.16%). It was statistically significant over rest of the botanicals. The Tulsi leaf extract (43.00 mm and 52.22%) was statistically at par with Garlic clove extract which recorded 47.33 mm colony diameter and 47.41% inhibition. Karanj leaf extracts which recorded minimum colony diameter which was 66.00 mm and 26.66% mycelial growth inhibition. The untreated control plates recorded maximum colony diameter *i.e.;* (90.00 mm).

At 20% concentration out of seven botanicals tested the Turmeric Rhizome extract was found to be most effective and recorded least mycelial growth (11.00 mm) with highest mycelial inhibition (87.77%) of *Colletotrichum capsici* and statistically significant over rest of the botanicals followed by Neem leaf extract which recorded mean colony diameter and percent mycelial inhibition of 17.00 mm and (81.11%) and statistically significant over rest of the botanicals. Garlic bulb extract recorded colony diameter of 20.00 mm and corresponding inhibition of 77.77% and statistically at par with Tulsi leaf extract (29.32 mm and 67.42%). It was statistically significant over rest of the botanicals. The Ginger rhizome extract (31.33 mm and 65.18%) was statistically at par with Onion bulb extract which recorded 33.00 mm colony diameter and 63.33 inhibition. Karanj leaf extracts which recorded minimum colony diameter which was
45.66 mm and 49.26% mycelial growth inhibition. The untreated control plates recorded maximum colony diameter *i.e.;* (90.00 mm).

Average mycelial growth least mycelial growth recorded by Neem leaf extract (19.00 mm) with highest percent mycelial inhibition (78.88%) of *Colletotrichum capsici* over rest of the botanicals followed by Turmeric rhizome extract which recorded mean colony diameter and percent mycelial

inhibition of 20.50 mm and 77.21% and statistically significant over rest of the botanicals. Garlic bulb extract recorded colony diameter of 33.66 mm and corresponding inhibition of 62.59% and statistically at par with onion bulb extract (34.83 mm and 61.29%). It was statistically significant over rest of the botanicals. The Tulsi leaf extract and Ginger Rhizome extract recorded same average mycelial growth and percent inhibition (36.16 mm and 59.81%). Karanj leaf extracts which recorded minimum average colony diameter which was 55.83 mm and 37.96% mycelial growth inhibition.

Summary and Conclusions

- All the two contact and six systemic fungicides evaluated *in vitro* were found fungistatic against *Colletotrichum capsici.* Among all these fungicides, Tricyclazole was found to be most effective with significantly maximum mycelial growth inhibition and least mycelial growth followed by fungicides *viz.* Azostrobin, Tebuconazole, Difenconazole, Propiconazole, Carbendazim + Mancozeb. While comparatively minimum average radial mycelial growth inhibition was recorded with Hexaconazole followed by Propineb
- All the seven fungal and one bacterial antagonists evaluated *in vitro* were found antifungal against *Colletotrichum capsici* exhibited significant mycelial growth inhibition. However, *Trichoderma asperallum, Pseudomonas fluorescens, Trichoderma harzianum and Gliocladium virens* recorded significantly highest mycelial growth inhibition. Rest of the bioagents, *Trichoderma ligronum, Trichoderma koningii.*

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