

Management of *Cercospora* Leaf Spot in Mungbean by use of Botanicals, Fungicides and Bio-agents

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ABSTRACT: This study aimed to investigate the efficiency of Management of *Cercospora* leaf spot in mungbean by use of botanicals, fungicides and bio-agents.

The mungbean crop is infected by a large number of pathogens such as fungi, bacteria, virus and nematodes in which mungbean *Cercospora* Leaf Spot causes significantly losses. At present *Cercospora* Leaf Spot is being managed by using fungicide through seed and soil treatment. However, fungicides are more costly and pollutant to environment. Many plant extracts are known to have antifungal activity. During the present investigation, extract of 5 plants species namely, Neem, Garlic, Tulsi, Onion, Ginger were evaluated for fungi toxicity against *C. canescens* by using poison food technique.

The per cent inhibition in radial growth of *Cercospora canescens* was maximum in Garlic (85.22 %), followed by Tulsi (84.35 %), Onion (84.13 %), Ginger (83.48 %), Neem (72.61 %). The minimum growth is recorded in Neem (58.51), however Ginger and Onion, Tulsi, Onion and Tulsi, Garlic, Tulsi and Garlic were significantly at par to each other, Garlic was found significantly superior at 10 per cent concentration after 7 days of incubation. The efficacy of chemicals *in vitro* revealed that Carbendazim completely inhibited the mycelial growth of *C. canescens* at 200 ppm concentration. Bio-agents, *Trichoderma viride* and *Trichoderma harzianum* were evaluated by using dual culture technique against *Cercospora canescens* causing leaf spot of mungbean. The radial growth were (10.20 mm) and (19.30 mm) in *T. viride* and *T. harzianum*, respectively at 7 days of incubation which differed significantly from each other.

Keywords: Botanicals, Garlic, *Cercospora*, Bio-agent, Chemicals.

INTRODUCTION

Vigna unguiculata is one of the most important legume crops of the world. It has four cultivated subspecies of which two are well known, namely *unguiculata* (grain cowpea) and *sesquipedalis* (yardlong bean). Yardlong bean is mostly cultivated in Asia. Young edible pods of this crop are consumed in several ways, both raw and cooked forms. The young pods can be harvested for four to five times beginning at about 45–50 days after sowing. *Cercospora* leaf spot (CLS) disease is an important disease of grain cowpea and yardlong bean. This disease is caused by the fungi *Cercospora canescens* and *Pseudocercospora cruenta* resulting in yield loss of up to 40% (Schneider *et al.* 1976; Fery *et al.*, 1977). The leaf spot symptoms of *C. canescens* are circular, while those of *P. cruenta* are angular. Although *C. canescens* is comparatively a weaker parasite than *P. cruenta*, the former has a wider host range under tropical climates than the latter (Fery *et al.*, 1977). Average yield of the crop is very low mainly due to low inherent yield potential and susceptibility of the crop to diseases (Thakur *et al.*, 1977). Leaf spot disease caused by *Cercospora canescens* Ellis & Martin is a

serious disease in the mungbean growing areas of the country where high humidity prevails during the growing season (Bashir & Zubair 1985). *Cercospora* leaf spot is one of the important diseases that cause serious losses to mungbean crop and 23% losses in yield have been reported (Quebral & Cagampang 1970). Maximum loss of 61% was observed in case of grain yield (Iqbal *et al.*, 1995). The disease starts appearing about 30-40 days after planting. Depending upon the temperature and humidity, it spreads rapidly in susceptible varieties causing premature defoliation and reduction in size of pods and grains (Grewal *et al.*, 1980). Several workers had reported the effective control of the disease with the application of fungicides (Singh & Naik, 1977; Singh & Singh 1978). Mungbean [*Vigna radiata* (L.) Wilczek], is an indigenous vegetable legume and one of the most important pulse crops in Southeast Asia. Being rich in digestible protein (24%), mungbean is utilized in the cereal-based diets (Khattak *et al.*, 2003). It contains vitamin A (94 mg), iron (7.3 mg), zinc (3mg), calcium (124 mg) and folate (549 mg) per 100g dry seed. Usually it is used in split form (Dhal) and in other different food products (Rasul *et al.*, 2012). Fallow period of 70-90 days (April to

June) in rice wheat cropping system is suitable to raise a catch crop of mungbean. Being low input requiring, short duration, high value crop and restorative crop, mungbean, find its place in rice-wheat and other crop rotations (Achakzai *et al.*, 2012). Mungbean, being leguminous crop fixes nitrogen thereby improving soil fertility and requires comparatively lesser irrigation than various field crops (Khan *et al.*, 2008). One of the environmentally friendly approaches is applying a natural enemy of the pathogen or bio-fungicide. Phyllosphere fungi such as *Trichoderma* spp. has been reported as a natural enemy for particular plant pathogen (Baker 1987; Thakur and Harsh 2014). The initial symptoms of the disease appear as water soaked spot on leaves. As spots become older may coalesce together, causing enlarged dead area on the infected leaves. Heavy infections of *Cercospora* can cause premature defoliation of the mungbean plant. Sometimes the leaves may become malformed and wrinkled. Maturity is delayed in the diseased plants resulting poor pod formation. Seeds that developed on severely infected plants are small and immature (Poehlman, 1991). Different approaches such as spray of chemical fungicides (Singh and Singh 1978), spray of different plant extracts and use of resistant variety are tried to control *Cercospora* leaf spot of mungbean. The extracts were prepared by using the method of (Asrafuzzaman and Hossain 1992). After washing weighted leaf, the big leaves were cut into small pieces. For getting extract, weighted plant parts were blended and then distilled water was added into the jug of the blender. The pulverized mass was squeezed through 3 folds of fine cotton cloth. For getting 1:4 (w/v) ratio 400 ml of distilled water was added with 100 g of plant parts, 1:5 (w/v) ratio 500 ml of distilled water was added with 100 g of plant parts, 1:6 (w/v) ratio 600 ml of distilled water was added with 100 g of plant parts.

Plant extracts were applied in the field as foliar spray. Spraying was done 3 times at 7 days interval starting from vegetative growth. The plants were naturally infected. The disease incidence and severity was recorded at 30, 40, 50 and 60 DAS (Days after Sowing). For disease incidence, the infected plants were identified by comparing it symptoms critically with those already published by (Ahmed 1985).

METHODS AND MATERIAL

For the management of cercospora disease, three experiments viz., evaluation of botanicals, chemicals and bio-agents were carried out at Acharya Narendra Deva University of Agriculture and Technology, Ayodhya (U.P.) Kharif-2019.

1. Efficacy of different plant extracts against

***Cercospora canescens in vitro*:** Efficacy of locally available 5 plants were selected for this study which have antifungal properties. The leaf/bulb extract of these plants were screened at 5 and 10 per cent concentration against *Cercospora canescens* following poison food technique. These plants were selected because they were associated with disease management practices in several parts of India. To obtain extracts, these plant parts were thoroughly washed under running clean tap water to remove dust and other foreign matter from surface. Then plant materials were weighed individually (100 g each). Materials were surface sterilized with 0.1% HgCl₂ solution and washed thrice with sterilized water. Each plant part was grinded individually in Pestle and Mortar by adding equal amount (100 ml) of sterilized water (1:1 w/v) and boiled at 80°C for 10 minutes. The resultant slurry was strained through a double-layered muslin cloth to remove the uncrushed fibrous tissues of plant (Bdliya, & Alkali 2010).

Table 1: List of plants with common name, English name, botanical name, family and their part used.

Sr. No.	Common Name	English Name	Botanical Name	Family	Part Used
1.	Neem	Neem	<i>Azadirachta indica</i>	Meliaceae	Leaves
2.	Lahsun	Garlic	<i>Allium sativum</i>	Liliaceae	Bulb
3.	Pyaz	Onion	<i>Allium cepa</i>	Liliaceae	Bulb
4.	Adarakh	Ginger	<i>Zingiber officinale</i>	Zingiberaceae	Rhizome
5.	Tulsi	Ocimum	<i>Ocimum sanctum</i>	Lamiaceae	Leaves

All the plant extracts were tested at 5 and 10 per cent concentration under *in vitro* condition by using poison food technique to study the inhibitory effect of these botanical on mycelial growth of *C. canescens*. 5 and 10 ml plant extract of each stock solution were added to the 92.5, 90.0 and 85 ml of sterilized PDA medium.

This poisoned PDA poured into sterilized Petri dishes. Three replications were maintained for each concentration. After solidification of medium in Petri dishes, each plates was centrally inoculated with 5 mm disc of 7 days old culture cut from the edge of culture of *C. canescens* using sterilized cork borer. All the inoculated plates were incubated at 28 ± 2°C. Colony diameter was measured after 4 and 7 days of inoculation. Per cent inhibition over control was

calculated by applying the following formula (Vincent, 1927):

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

Where,

I = Per cent inhibition

C = Colony diameter in control (mm)

T = Colony diameter in treated (mm)

2. Efficacy of chemicals against *Cercospora*

***canescens in vitro*:** Efficacy of 2 fungicides were evaluated at 100, 200, and 500 ppm for studying their inhibitory effect on the mycelial growth of *Cercospora canescens* using poisoned food technique (Chaurasia *et al.*, 2003). The lists of fungicides used are given in (Table 2) for preparation of different concentration of fungicides were weighted with the help of electronic

balance. The weighted fungicides were added in sterilized PDA. This PDA are poured in sterilized Petri dishes. The per cent inhibition over control was calculated by earlier described formula.

3. Efficacy of bio-agents against *Cercospora canescens* in vitro: Two bio-agents were used *Trichoderma viride* and *Trichoderma harzianum* against *Cercospora canescens* which obtained from the Department of Plant Pathology, ANDUAT, Kumarganj, Ayodhya (U.P.) were assessed by using dual culture technique by measuring the radial growth of *C. canescens* as well as that of *Trichoderma* spp.

Table 2: List of fungicides, trade name, chemical name and their source of supply.

Sr. No.	Common Name	Trade Name	Chemical Name	Source
1.	Carbendazim	Bavistin 50 W.P.	Methyl 11, 2-Benzimidazole-2yl carbamate	BASF, india Ltd.
2.	Thiram	Thiram 75 W.P.	Tetramethylthiuram di-sulphide	Bharat Pulverising Mills, Ltd.

Table 3: List of bio-agents used in management of cercospora.

Sr. No.	Name of bio-agents
1.	<i>Trichoderma viride</i>
2.	<i>Trichoderma harzianum</i>

Twenty ml of sterilized melted PDA was aseptically poured in sterilized Petri dishes (90 mm diameter) and allowed to solidify. Five mm disc of each antagonist and *C. canescens* cut with the help of sterilized cork borer from the age of three days old culture and were placed in Petri dishes having solidified PDA in such a

manner that they lie opposite to each other 60 mm apart in four replication. In check Petri dishes were inoculated only with *C. canescens* bits. These Petri dishes were kept in BOD incubator at 26 ± 2 °C.

And the extent of interaction was observed by measuring area covered by in dual culture and in the control at 4 and 7 days of incubation. The per cent inhibition of the interacting fungi was calculated as follows:

$$\text{Per cent growth inhibition} = \frac{A_1 - A_2}{A_1} \times 100$$

Whereas,

A_1 = Area covered by the *C. canescens* in control

A_2 = Area covered by the *C. canescens* in dual culture

1 (a). Effect of plant extract at different concentrations against *Cercospora canescens* on mycelial growth in vitro at 4 & 7 days:

(i) At 4 days of incubation:

In 5 per cent concentration minimum radial growth was obtained in Garlic (5.30 mm) followed by Tulsi (5.90 mm), Onion (6.70 mm), Ginger (7.20 mm) and Neem (10.10 mm) as compared to control (23.00). Each treatment significantly superior to control. Garlic, Tulsi, Onion, and Ginger were significantly at par to each other's; however Neem was statistically different to other treatments (Table 4) (Devi *et al.*, 2013).

The radial growth ranged from (3.40 mm) to (3.80 mm) in 10 per cent concentration. Similar pattern were found as 5 per cent concentration and every treatment significantly superior to control. Garlic, Tulsi, Onion, and Ginger were significantly at par to each other, however Neem was statistically different to other treatments (Table 4).

Table 4: Effect of plant extract at different concentrations against *C. canescens* on mycelial growth in vitro at 4 days.

Plant extract	Mycelial growth (mm)	
	Concentration (%)	
	5 %	10 %
Neem (leaf)	10.10	6.30
Garlic (bulb)	5.30	3.40
Tulsi (leaf)	5.90	3.60
Onion (bulb)	6.70	3.65
Ginger (rhizome)	7.20	3.80
Control	23.0	23.00
CD at 5 %	2.74	1.26
SEm ±	0.43	0.16

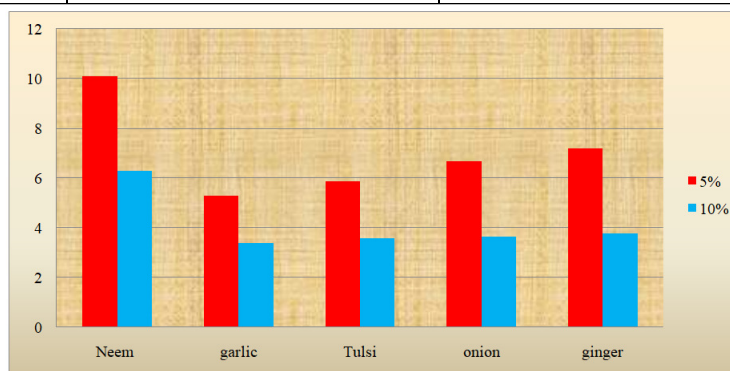


Fig. 1. Effect of plant extract at different concentrations against *Cercospora canescens* on mycelial growth in vitro at 4 days.

The minimum radial growth was obtained in 5 and 10 percent concentrations in Garlic followed by Tulsi, Onion, Ginger, Neem at 4 days of incubation.

(ii) At 7 days of incubation: In 5 per cent concentration minimum radial growth was obtained in Garlic (8.00 mm) followed by Tulsi (8.90 mm), Onion (10.00 mm), Ginger (10.80 mm), Neem (15.10 mm), compared to control (42.00 mm), Each treatment significantly superior to control. Garlic, Tulsi, Onion, and Ginger were significantly at par to each other; however Neem was statistically different to other treatments (Table 5).

The similar results were obtained in 10 per cent concentration as 5 per cent concentration and radial growth ranged from 5.10 mm to 9.50 mm. Each treatment significantly superior to control. Garlic, Tulsi, Onion, and Ginger were significantly at par to each other; however Neem was statistically different to other treatments (Table 5). The minimum radial growth was obtained in 5 and 10 percent concentrations in Garlic

followed by Tulsi, Onion, Ginger, Neem at 7 days of incubation.

1 (b) Efficacy of plant extract against *Cercospora canescens* on per cent inhibition:

(i) At 4 days of incubation: In 5.0 per cent concentration in the maximum per cent inhibition in mycelia growth of *Cercospora canescens* was recorded in Garlic (76.96 %) followed by Tulsi (74.35 %), Onion (70.87 %), Ginger (68.70 %), Neem (56.09 %). The minimum growth is recorded in Neem (48.50), however Garlic, Tulsi, Onion, and Ginger were significantly at par to each other, Garlic was found significantly superior all the treatments (Table 6) (Islam *et al.*, 2006). The similar patterns were obtained in 10.0 per cent concentration and per cent inhibition ranged from (80.95) to (64.05) per cent. All treatments significantly superior to control. Garlic was found statistically superior all the treatments, however Garlic, Tulsi, Onion, and Ginger were significantly at par with each other, while Neem was statistically different to other treatments (Table 6) (Hossain and Hossain 2014).

Table 5: Effect of plant extract at different concentrations against *Cercospora canescens* on mycelial growth in vitro at 7 days.

Plant extract	Mycelial growth (mm)	
	Concentration (%)	
	5.00 %	10.00 %
Neem (leaf)	15.10	9.50
Garlic (bulb)	8.00	5.10
Tulsi (leaf)	8.90	5.40
Onion (bulb)	10.00	5.50
Ginger (rhizome)	10.80	5.70
Control	42.00	42.00
CD at 5 %	2.74	1.26
SEm ±	0.89	0.41

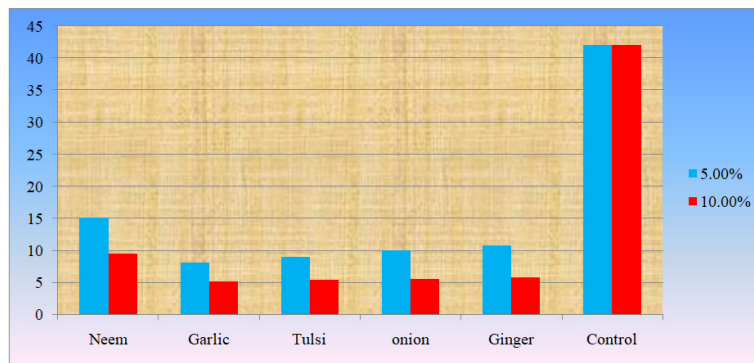


Fig. 2. Effect of plant extract at different concentrations against *Cercospora canescens* on mycelial growth in vitro at 7 days.

Table 6: Effect of plant extract at different concentrations against *Cercospora canescens* on percent inhibition in vitro at 4 days.

Plant extract	Percent inhibition	
	Concentration (%)	
	5.00 %	10.00 %
Neem (leaf)	56.09 (48.50)	64.05 (53.29)
Garlic (bulb)	76.96 (61.33)	80.95 (64.27)
Tulsi (leaf)	74.35 (59.59)	78.81 (62.73)
Onion (bulb)	70.87 (57.40)	76.19 (60.82)
Ginger (rhizome)	68.70 (56.07)	74.29 (59.54)
Control	0.00	0.00
CD at 5 %	4.50	6.30
SEm ±	1.46	2.04

Figure given in parenthesis are transformed value

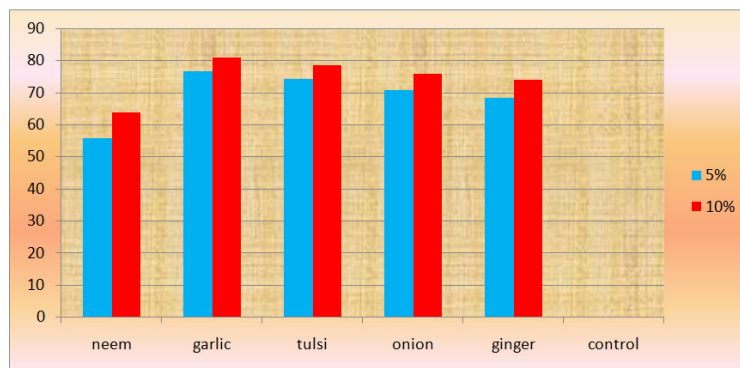


Fig. 3. Effect of plant extract at different concentrations against *Cercospora canescens* on percent inhibition in vitro at 4 days.

The maximum per cent inhibition in mycelial growth of *C. canescens* was obtained in 5.0 and 10.0 per cent concentration in Garlic followed by Tulsi, Onion, Ginger and Neem after 4 days incubation.

(ii) At 7 days of incubation

In 5.0 per cent concentration the maximum per cent inhibition in mycelial growth of *C. canescens* was recorded in Garlic (85.22 %), followed by Tulsi (84.35 %), Onion (84.13 %), Ginger (83.48 %), Neem (72.61 %). The minimum growth is recorded in Neem (58.51), however Garlic, Tulsi, Onion, and Ginger were

significantly at par to each other while Garlic was found significantly superior all the treatments (Table 7). (Uddin *et al.*, 2013).

Similar, patterns were obtained in 10.0 per cent concentration and per cent inhibition in mycelial growth of *C. canescens* ranged from 87.86 to 77.38 per cent, all treatments were significantly superior to control. The minimum growth is recorded in Neem (61.63), however Garlic, Tulsi, Onion, and Ginger were significantly at par to each other while Garlic was found significantly superior all the treatments (Table 7).

Table 7: Effect of plant extract at different concentrations against *C. canescens* on per cent inhibition in vitro at 7 days.

Plant extract	Per cent inhibition	
	Concentration (%)	
	5.00 %	10.00 %
Neem (leaf)	72.61 (58.51)	77.38 (61.63)
Garlic (bulb)	85.22 (67.48)	87.86 (69.67)
Tulsi (leaf)	84.35 (66.73)	87.14 (69.08)
Onion (bulb)	84.13 (66.54)	86.90 (69.19)
Ginger (rhizome)	83.48 (66.05)	86.43 (69.07)
Control	0.00	0.00
CD at 5 %	3.76	7.38
SEM ±	1.22	2.40

Figure given in parenthesis are transformed value

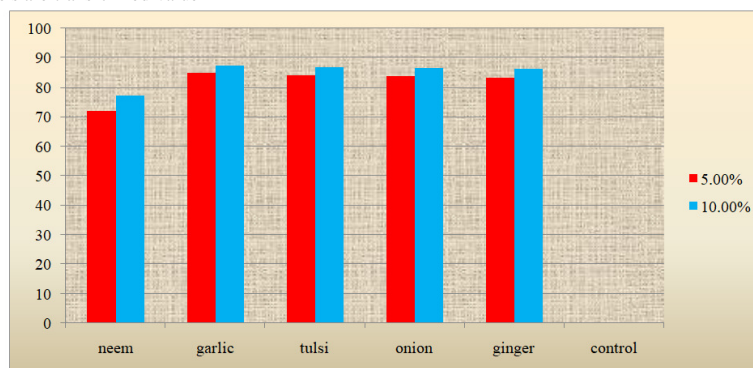


Fig. 4. Effect of plant extract at different concentrations against *C. canescens* on per cent inhibition in vitro at 7 days.

The maximum per cent inhibition in mycelial growth of *C. canescens* was obtained in 5.0 and 10.0 per cent concentration in Garlic followed by Tulsi, Onion, Ginger, Neem after 7 days incubation.

2 (a) Efficacy of chemicals at different concentration against *Cercospora canesc* on mycelial growth in vitro.

Two fungicides *viz.*, Carbendazim, Thiram used in present studies were evaluated *in vitro* against *C. canescens* by poison food technique at 100, 200, and 500 ppm concentration after 4 and 7 days of incubation.

(i) At 4 days of incubation. Results presented in (Table 08) and (Fig. 5) showed that in 100 ppm concentration radial growth was zero obtained in Carbendazim

followed by minimum radial growth in Thiram (18.00 mm) as compared to control (23.00 mm). Each treatment was significantly superior to control. Carbendazim were statistically differ to Thiram, however Carbendazim was found superior to Thiram. The similar results were found in 200 ppm as 100 ppm. The radial growth ranged from 0 to 23.00 mm. Each treatment significantly superior to control. Carbendazim was statistically differ to Thiram, however

Carbendazim was found superior to Thiram (Table 8 and Fig. 5).

The radial growth ranged from 0 to 23.00 mm in 500 ppm concentration. Similar pattern were found as 200 ppm concentration and every treatments significantly superior to control. Carbendazim was statistically differ to Thiram, however Carbendazim was found superior to Thiram (Table 8 and Fig. 5).

Table 8: Effect of chemicals at different concentrations against *Cercospora canescens* on mycelial growth in vitro at 4 days

Chemicals name	Mycelial growth (mm)		
	Concentration (ppm)		
	100 ppm	200 ppm	500 ppm
Carbendazim	0.00	0.00	0.00
Thiram	18.00	16.00	16.00
Control	23.00	23.00	23.00
CD at 5%	0.72	1.65	1.65
SEm ±	0.21	0.48	0.48

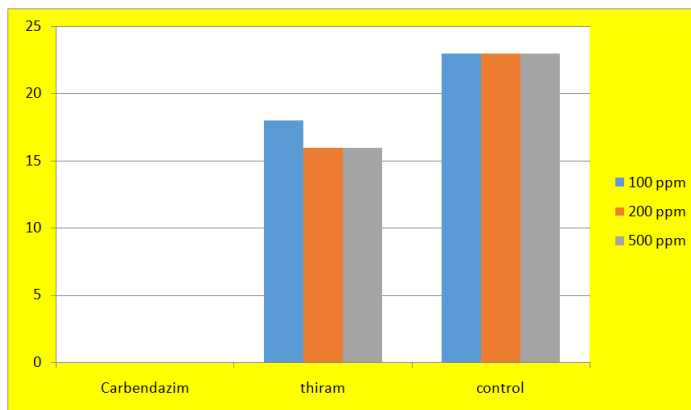


Fig. 5. Effect of chemicals at different concentrations against *Cercospora canescens* on mycelial growth in vitro at 4 days.

(ii) **At 7 days of incubation.** In 100 ppm concentration zero radial growth was recorded in Carbendazim followed the minimum radial growth was obtained in Thiram (29.20 mm) as compared to control (42.00 mm). Every treatment was significantly superior over to control. Carbendazim was statistically differ to Thiram, however Carbendazim was found superior to Thiram (Table 9 and Fig. 6).

The similar results were obtained in 200 ppm and 500 ppm concentration as 100 ppm concentration and the radial growth was both concentration ranged from 0.0 to 42.00 mm. Each treatments were significantly superior over to control. Carbendazim was statistically differ to Thiram, however Carbendazim was found superior to Thiram (Table 9 and Fig. 6).

Table 9: Effect of chemicals at different concentrations against *Cercospora canescens* on mycelial growth in vitro at 7 days.

Chemicals name	Mycelial growth (mm)		
	Concentration (ppm)		
	100 ppm	200 ppm	500 ppm
Carbendazim	0.00	0.00	0.00
Thiram	29.20	27.00	23.00
Control	42.00	42.00	42.00
CD at	1.22	3.00	2.98
SEm ±	0.35	0.87	0.86

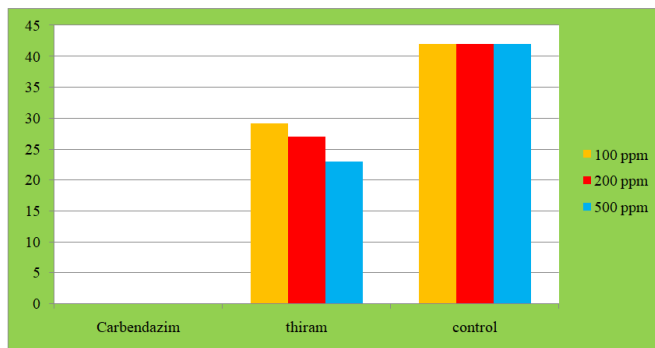


Fig. 6. Effect of chemicals at different concentrations against *Cercospora canescens* on mycelial growth in vitro at 7 days.

2 (b). Efficacy of chemicals on percent inhibition against *Cercospora canescens*

(i) At 4 days of incubation. In 100 ppm concentration complete (100 %) per cent inhibition in mycelial

growth of *C. canescens* was recorded in Carbendazim followed by Thiram (21.74 %) Carbendazim was statistically differ to Thiram, however Carbendazim was found superior than Thiram (Table 10 and Fig. 7).

Table 10: Effect of chemicals at different concentrations against *Cercospora canescens* on per cent inhibition in vitro at 4 days.

Chemicals name	Per cent inhibition		
	Concentration (ppm)		
	100 ppm	200 ppm	500 ppm
Carbendazim	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Thiram	21.74 (27.79)	30.00 (33.21)	36.96 (37.44)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD at 5%	0.09	0.11	0.44
SEm ±	0.30	0.38	0.13

Figure given in parenthesis are transformed values

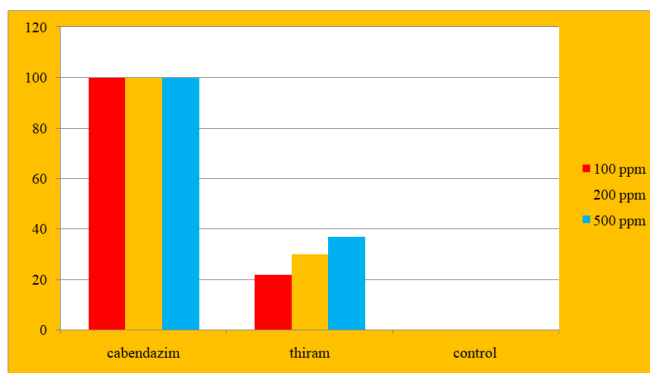


Fig. 7. Effect of chemicals at different concentrations against *Cercospora canescens* on per cent inhibition in vitro at 4 days.

The similar patterns were recorded in 200 and 500 ppm concentration as 100 ppm concentration ranged from 30.00 to 100.00 per cent and 36.96 to 100.00 per cent, respectively. Each treatment in both concentrations were significantly superior to control. While Carbendazim was statistically differ to Thiram, however Carbendazim was found superior to Thiram (Table 10 and Fig. 7).

(ii) At 7 days of incubation. In 100 ppm concentration Carbendazim completely (100.00 %) inhibited the mycelial growth followed by Thiram (30.48 %). Carbendazim was statistically differ to Thiram, however Carbendazim was found superior to Thiram (Table 11 and Fig. 8).

Table 11: Effect of chemicals at different concentrations against *C. canescens* on per cent inhibition *in vitro* at 7 days.

Chemicals name	Percent inhibition		
	Concentration (ppm)		
	100 ppm	200 ppm	500 ppm
Carbendazim	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Thiram	30.48 (33.51)	35.71 (36.70)	45.24 (42.27)
Control	00.00	00.00	00.00
CD at 5%	0.38	0.43	0.52
SEm ±	0.11	0.12	0.15

Figure given in parenthesis are transformed values

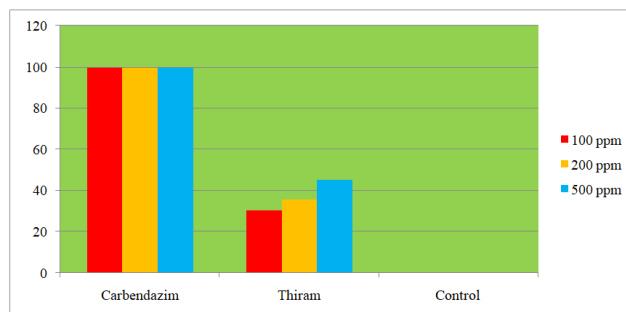


Fig. 8. Effect of chemicals at different concentrations against *C. canescens* on per cent inhibition *in vitro* at 7 days.

The similar patterns were obtained in 200 and 500 ppm concentration as 100 ppm concentration and the per cent inhibition in radial growth were ranged from 435.71 to 100.00 per cent and 45.24 to 100.00 per cent, respectively, each treatment was significantly superior to control. Carbendazim was statistically differ to Thiram, however Carbendazim was found superior to Thiram (Table 11 and Fig. 8).

3. Efficacy of bio-agents against *Cercospora canescens* on radial growth and per cent inhibition using dual culture technique *in vitro*

The efficacy of two bio-agent *Trichoderma viride* and *Trichoderma harzianum* were tested for radial growth

and percent inhibition of *Cercospora canescens* by using dual culture technique.

(i) At 4 days of incubation. The radial growth was recorded maximum in *Trichoderma harzianum* (12.80 mm) followed by *Trichoderma viride* (7.50 mm). The radial growth in control was (23.0 mm). The radial growth significantly differed with each other in all the treatments after 4 days of incubation.

Trichoderma viride showed maximum per cent growth inhibition (67.39 %) followed by *Trichoderma harzianum* (44.35 %) at 4 days of incubation, respectively which is significantly differed with each other (Table 12 and Fig. 09).

Table 12: Effect of bio-agents against *Cercospora canescens* on radial growth and Growth Inhibition using dual culture technique after 4 days incubation.

Fungal antagonism	Mycelial growth (mm)	Growth inhibition (%)
<i>Trichoderma viride</i>	7.50	67.39 (55.19)
<i>Trichoderma harzianum</i>	12.80	44.35 (41.75)
Control	23.00	0.00 (0.00)
SEm±	0.32	0.54
CD at 5%	1.11	1.88

Figure given in parenthesis are transformed value

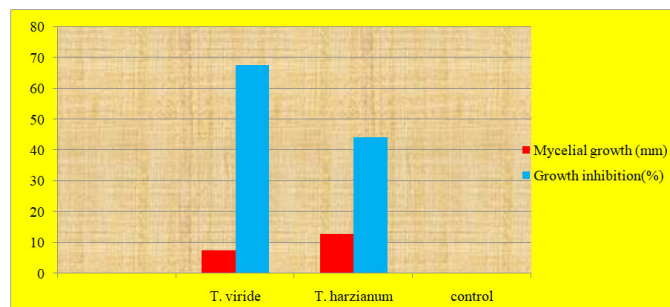


Fig. 9. Effect of bio-agents against *Cercospora canescens* on radial growth and growth Inhibition using dual culture technique after 4 days incubation.

(ii) **At 7 days incubation.** The similar result were obtained at 7 days of incubation, however, the radial growth were *Trichoderma harzianum* (19.30 mm) and *Trichoderma viride* (10.20 mm). The radial growth in control was (42.0 mm). The radial growth significantly differed with each other in all the treatments after 7 days of incubation (Table 13). *Trichoderma viride* showed maximum per cent inhibition (75.71 %)

followed by *Trichoderma harzianum* (54.05 %) after 7 days of incubation, respectively which is significantly differed with each other (Table 13 and Fig. 10). Thus, it is very clear that *Trichoderma viride* was better in reducing radial growth of *Cercospora canescens* as compared to *Trichoderma in vitro*.

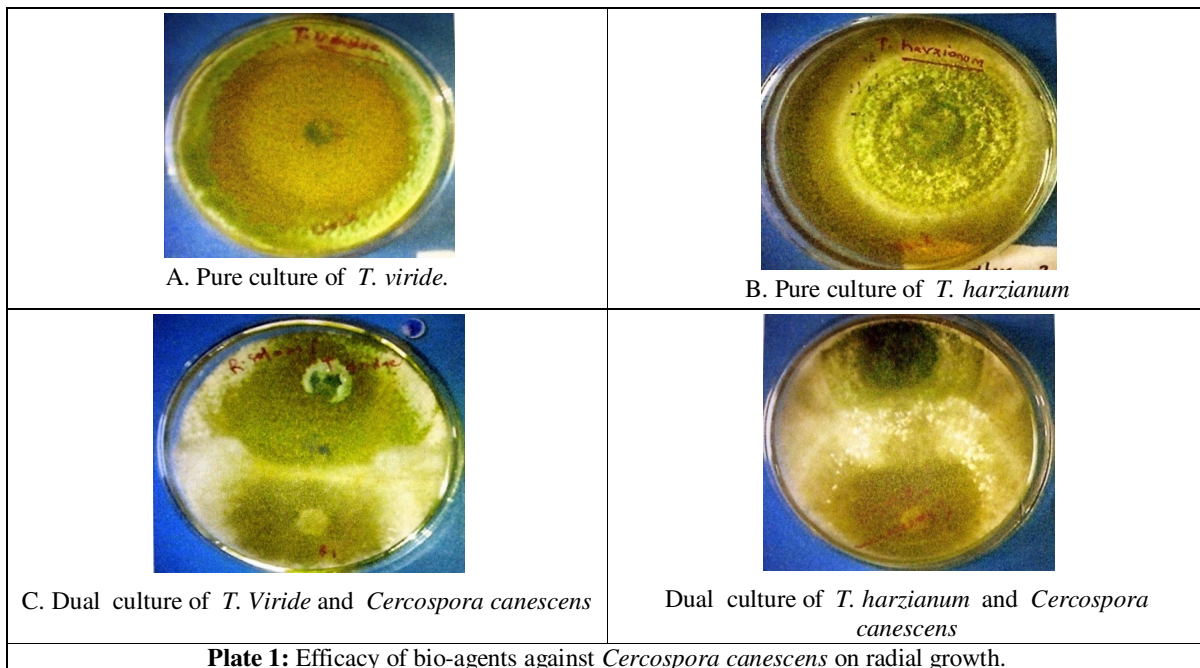


Table 13: Effect of bio-agents against *Cercospora canescens* on radial growth and growth inhibition using dual culture technique after 7 days incubation.

Fungal antagonism	Mycelial growth (mm)	Growth inhibition (%)
<i>Trichoderma viride</i>	10.20	75.71 (47.32)
<i>Trichoderma harzianum</i>	19.30	54.05 (35.97)
Control	42.00	0.00 (0.00)
SEm±	1.22	1.31
CD at 5%	4.23	4.53

Figure given in parenthesis are transformed value

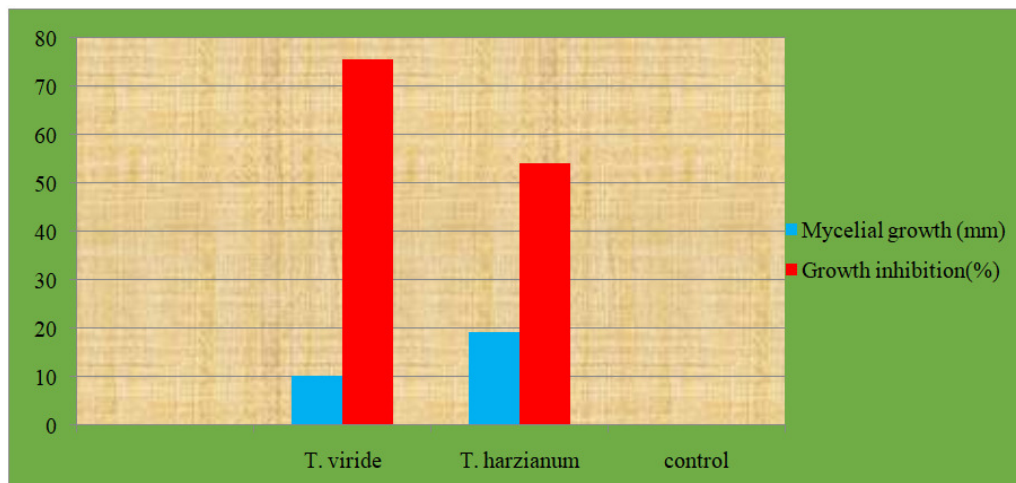


Fig. 10. Effect of bio-agents against *Cercospora canescens* on radial growth and growth inhibition using dual culture technique after 7 days incubation.

RESULT AND DISCUSSION

Efficacy of botanicals against *Cercospora canescens* *in vitro*

In vitro: There is an all-round compulsion to go is far bio rational alternatives, as the use of fungicides for disease management are known to pollute the environment, soil and water resulting deleterious effect of human health and biosphere. During the present investigation, extract of 5 plants species namely, Neem, Garlic, Tulsi, Onion, Ginger were evaluated for fungi toxicity against *C. canescens* by using poison food technique.

Effect of plant extract at different concentrations against *C. canescens* on mycelial growth *in vitro* at 4 & 7 days of incubation.

(i) At 4 days of incubation: In 5 per cent concentration minimum radial growth was obtained in Garlic (5.30 mm) followed by Tulsi (5.90 mm), Onion (6.70 mm), Ginger (7.20 mm) and Neem (10.10 mm) as compared to control (23.00). Each treatment significantly superior to control. Garlic, Tulsi, Onion, and Ginger were significantly at par to each other, however Neem was statistically different to other treatments (Table 4).

The radial growth ranged from (3.40 mm) to (3.80 mm) in 10 per cent concentration. Similar pattern were found as 5 per cent concentration and every treatment significantly superior to control. Garlic, Tulsi, Onion, and Ginger were significantly at par to each other, however Neem was statistically different to other treatments (Table 4).

The minimum radial growth was obtained in 5 and 10 percent concentrations in Garlic followed by Tulsi, Onion, Ginger, Neem at 4 days of incubation.

(ii) At 7 days of incubation: In 5 per cent concentration minimum radial growth was obtained in Garlic (8.00 mm) followed by Tulsi (8.90 mm), Onion (10.00 mm), Ginger (10.80 mm), Neem (15.10 mm), compared to control (42.00 mm), Each treatment significantly superior to control. Garlic, Tulsi, Onion, and Ginger were significantly at par to each other, however Neem was statistically different to other treatments (Table 5).

The similar results were obtained in 10 per cent concentration as 5 per cent concentration and radial growth ranged from 5.10 mm to 9.50 mm. Each treatment significantly superior to control. Garlic, Tulsi, Onion, and Ginger were significantly at par to each other, however Neem was statistically different to other treatments (Table 5).

Efficacy of plant extract against *Cercospora canescens* on per cent inhibition:

(i) At 4 days of incubation: In 5.0 per cent concentration in the maximum per cent inhibition in mycelia growth of *Cercospora canescens* was recorded in Garlic (76.96 %) followed by Tulsi (74.35 %), Onion (70.87 %), Ginger (68.70 %), Neem (56.09 %). The minimum growth is recorded in Neem (48.50), however Garlic, Tulsi, Onion, and Ginger were significantly at par to each other, Garlic was found significantly superior all the treatments (Table 6).

The similar patterns were obtained in 10.0 per cent concentration and per cent inhibition ranged from (80.95) to (64.05) per cent. All treatments significantly superior to control. Garlic was found statistically superior all the treatments, however Garlic, Tulsi, Onion, and Ginger were significantly at par with each other, while Neem was statistically different to other treatments (Table 6).

The maximum per cent inhibition in mycelial growth of *C. canescens* was obtain in 5.0 and 10.0 per cent concentration in Garlic followed by Tulsi, Onion, Ginger and Neem after 4 days incubation.

(ii) At 7 days of incubation. In 5.0 per cent concentration the maximum per cent inhibition in mycelial growth of *C. canescens* was recorded in Garlic (85.22 %), followed by Tulsi (84.35 %), Onion (84.13 %), Ginger (83.48 %), Neem (72.61 %). The minimum growth is recorded in Neem (58.51), however Garlic, Tulsi, Onion, and Ginger were significantly at par to each other while Garlic was found significantly superior all the treatments (Table 7).

Similar, patterns were obtained in 10.0 per cent concentration and per cent inhibition in mycelial growth of *C. canescens* ranged from 87.86 to 77.38 per cent, all treatments were significantly superior to control. The minimum growth is recorded in Neem (61.63), however Garlic, Tulsi, Onion, and Ginger were significantly at par to each other while Garlic was found significantly superior all the treatments (Table 7). The maximum per cent inhibition in mycelial growth of *C. canescens* was obtained in 5.0 and 10.0 per cent concentration in Garlic followed by Tulsi, Onion, Ginger, Neem after 7 days incubation.

Kapadiya *et al.* (2001) also evaluated 12 plants extracts against *Cercospora canescens* under *in vitro* condition Custard apple, Arduci, Congress grass, Datura, Neem and Garlic were found effective in inhibiting the radial growth of the pathogen.

Hedge *et al.* (2002) found that extract of Neem, Ocimum, Onion, Bougainvillea, and Lantana inhibited the spore germination of *C. zinniae*. Bdiya and Akali (2010) reported that extract of Neem (seed), Garlic (clove), Onion (bulb), Ginger (rhizome) and Pawpaw (leaf) significantly reduce, the incidence and severity of *Cercospora* leaf spot of groundnut.

Efficacy of chemicals *in vitro*

In vitro: Use of chemicals for the management of disease caused by *C. canescens* is a practical approach and in some instances had become the principal method for the management.

Two different fungicides *viz.*, Carbendazim and Thiram were evaluated against *Cercospora canescens in vitro*. Carbendazim completely inhibited the mycelial growth of *Cercospora canescens* at 100 ppm concentration. However, Thiram provided the some growth at 500 ppm concentration. Singh and Singh (1976) found that Carbendazim most effective in controlling the disease followed by Copper Oxchloride and Mancozeb. The same results were also found Ahmad (1985); Jamadar and Padagnur (1995); Mian *et al.* (2000); Khalil and Jalaluddin (2004). Khander *et al.* (1986) also found Carbendazim was inhibitory to mycelial growth, however, Copper Oxchloride and Carbendazim were

inhibited the spore germination. This is the confirmation to present finding (Khalil and Jalaluddin 2004).

However, under field condition Topsin-M and Carbendazim were found effective in reducing disease incidence and increase grain yield (Elazagus and Mew 1987; Kaur *et al.*, 2004).

Saxena and Tripathi (2006) also found that Mancozeb, Bavistin, Topsin-M, Kavach and Propicoazole were significantly reduced the disease severity. The same results were also reported by Singh *et al.* (2007); Rathore (2006); Dubey and Singh (2006).

Efficacy of bio-agents *in vitro*

***In vitro*:** Bio-agents, *Trichoderma viride* and *T. harzianum* were evaluated by using dual culture technique against *Cercospora canescens* causing leaf spot of mungbean, The radial growth were (10.20 mm) and (19.30 mm) in *T. viride* and *T. harzianum*, respectively at 7 days of incubation which differed significantly from each other.

The per cent inhibition was also higher in *T. viride* (75.71 %) as compared to *T. harzianum* (54.05 %) after 7 days of incubation which differed significantly from each other.

Similar results were also obtained after 4 days of incubation with respect to radial growth and per cent inhibition. It seems that *T. viride* was better in inhibiting the radial growth as compared to *T. harzianum*.

Dubey and Patel (2001); Dubey (2002) also reported that *T. viride* was better in inhibiting radial growth of *Cercospora canescens* as compared to *T. harzianum* *in vitro* which support the present findings. However, Meena *et al.* (2002) and Khan & Sinha (2007) found *T. harzianum* was better in inhibiting the mycelial growth of *Cercospora canescens* as compared to *T. viride*. Upma Singh (2015) an extensive survey of Kota district resulted several fungal diseases occurring on *Trigonella* crop. Leaf spot caused by *Cercospora traversiana* was found to be more prominent and caused a great loss to the crop. To control the disease *Trichoderma* sps. *Viz.*, *Trichoderma viride*, *Trichoderma harzianum* were experimented *in vitro*.

CONCLUSION

1. The efficacy of botanicals Five plant extracts *viz.*, Neem, Garlic, Tulsi, Onion, Ginger were tested *in vitro* against *Cercospora canescens*. Food poison technique.

All the five plant extracts showed significant reduction in radial growth of test fungus at different concentrations (5.00 and 10.00 %). The affectivity of extracts increased with an increase in concentration and time of incubation (4 and 7 days).

(a) At 10.00 per cent concentration minimum radial growth was obtained in Garlic (5.10 mm) followed by Tulsi (5.40 mm), Onion (5.50 mm), Ginger (5.70 mm), Neem (9.50 mm), compared to control (42.00 mm), Each treatment significantly superior to control. Garlic and Tulsi, Onion, Tulsi and Onion, Ginger, Onion and Ginger were significantly at par to each other, however Ginger and Neem statistically different to each other.

(b) The per cent inhibition in radial growth of *C. canescens* was maximum in Garlic (85.22 %), followed

by Tulsi (84.35 %), Onion (84.13 %), Ginger (83.48 %), Neem (72.61 %). The minimum growth is recorded in Neem (58.51), however Ginger and Onion, Tulsi, Onion and Tulsi, Garlic, Tulsi and Garlic were significantly at par to each other, Garlic was found significantly superior at 10 per cent concentration after 7 days of incubation.

The similar trends were also observed in case of 5 per cent concentration after 4 days of incubation.

2. The efficacy of chemicals *In vitro* During assessment of two fungicides namely, Carbendazim and Thiram and against *C. canescens* revealed that Carbendazim completely inhibited the mycelial growth of *C. canescens* at 200 ppm concentration. while the 500 ppm concentration radial growth were completely inhibited, however Thiram provided the some growth at 500 ppm concentration.

3. The efficacy of bio-agents *Trichoderma viride* and *T. harzianum* were tested *In vitro* for growth and per cent inhibition of *C. canescens*. By using dual culture technique. *T. viride* was more effective as compared to *T. harzianum* in inhibiting the mycelial growth of *C. canescens*. 4 and 7 days of incubation.

(a) The radial growth was minimum in *T. viride* (7.50 mm) following by *T. harzianum* (10.20mm) as compared to control (19.30 mm) which were statistically different from each other at 7 days of incubation

(b) *Trichoderma viride* showed (75.71 %) per cent inhibition as compared to *T. harzianum* (54.05 %) after 7 days of incubation which significantly differed to each other Similar results were also obtained after 4 days of incubation with respect to radial growth and per cent inhibition.

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Conflict of Interest. None.

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