Plant Archives Vol. 26, No. 1, 2026 pp. 121-128 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.1.016

EVALUATION OF FUNGICIDES, PHYTO-EXTRACTS AND BIO-AGENTS AGAINST *CERCOSPORA CANESCENS* UNDER *IN VITRO* CONDITIONS

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(Date of Receiving-09-11-2025; Date of Revision-15-12-2025; Date of Acceptance-29-01-2026)

ABSTRACT

Effect of seven contact, systemic and combination fungicides were evaluated along with control at different concentrations *viz.,* 100, 250 and 500 ppm through poisoned food techniques against *Cercospora canescens*. Among the fungicides pyraclostrobin 13.3% + epoxyconazole 5% gave complete mycelial growth inhibition (100%) of at 100, 250 and 500 ppm concentrations followed by azoxystrobin 18.2% SC + difenoconazole 11.4% SC then carbendazim 25% WP + mencozeb 50% WP, hexaconazole 4% + zineb 68% WP, carbendazim 50 % WP, kersoximmethyl 44.3 SC. While, tebuconazole 25.9% EC was found less effective with inhibition of mycelial growth over control. Six plant extracts were evaluated at two concentrations (5 and 10%) under *in vitro* condition for their efficacy against *C. canescens* through Poisoned Food Technique and result revealed that the *Allium sativum* at 10 and 5 per cent concentration was found best in inhibiting the mycelial growth of *C. canescens* (77.96% and 68.55%) and found significantly superior over all the other plant extracts. While, datura leaf extract and Aak leaf extract was found less effective with inhibition of mycelial growth at 5 and 10 per cent concentrations. Efficacy of bio agents under *in vitro* conditions *Trichoderma harzianum* inhibited maximum mycelial growth (78.83%) against 789+3211+*Cercospora canescens.* While, minimum inhibition was recorded in bacterial bioagent *Pseudomonas fluorescens*.

***Key words*:** Mycelial growth, Plant extracts, Poisoned Food Technique,Inhibition, *Cercospora canescens.*

Introduction

Mothbean [*Vigna aconitifolia* (Jacq.) Marechal] is member of family: *Fabaceae*. It is rainfed *Kharif* pulse crop, also known as mat, moth, matki, Turkishgram or dew bean in its native country. It is hardy legume, widely used pulse crop in India's arid and semi-arid ecosystems (Sharma *et al*., 2014). In a variety of soil water shortage circumstances, mothbean is most practical and adaptable annual legume andcan withstand extra-evaporative stressors. (Kumar and Singh, 2002). It is able to grow with 200–300 mm annually rainfall and some yield has been reported at 50–60 mm per year low rainfall levels. Fertilizer applications to mothbean are uncommon in India. It's biological nitrogen fixation ranging from 28-65 kg/ha in the soil (Swain *et al.,* 2023). It has high nutritionalvalue as reservoir of high protein content (22-26%) and essential minerals like Mn, Ca, Zn & Fe (Sharma and Ratnoo, 2014). The composition of mothbean seeds is as protein 25.66%, moisture 10.30%, fat 1.4-1.5%, mineral matter 0.41%, carbohydrate 61.76% and fiber 3.90%. It is also a good source of high amount of amino acids like lysine (5.77%) and t ryptophan (3.23%) which is deficient in cereal crops (Satheand Venkatachalam, 2007). Mothbean is the principal in gredient of a famous product Bikaneri bhujiya. Mature seeds of mothare raw material for numerous delightful confectioneries *viz.,* papad, bhujia, mangori, vada, kheech etc. which are frequently use a sregular snacks (Rajora *et al.,* 2009). It is generally cultivated in hot and dry habitats of Northern-Western parts of India. However, it is also grown in Pakistan and Myanmar and other parts of Africa, Asia, United States, Australia, Cuba and Thailand (Bisht and Singh, 2013) and globally. India is key grower and producer with area of 13.19 lakh ha and 1,753 lakh tonnes production with productivity of 133 kg/ha (Anonymous 2022-23). In India, Rajasthan and Gujarat are the leading mothbean growing state (Sharma *et al.*, 2016). In Rajasthan, mothbean cultivation is prevalent in Bikaner, Barmer, Jodhpur, Jaisalmer and Churu districts. Rajasthan have major mothbean growing region as it covers nearly 86% area of the country (Gupta *et al.*, 2016), it occupies 10.44 lakh ha area with 0.22 million tonnes annual production with productivity of 270 kg/ha (Bhadkaria *et al.*, 2022). Mothbean infected by several types of fungal, bacterial and viral diseases. Mothbean is susceptible to many diseases, including cercospora leaf spot (*Cercospora canescens*), powdery mildew (*Erysiphe polygoni*), yellow mosaic virus (YMV), bacterial leaf spot (*Xanthomonas phaseoli*), dry root rot or charcoal rot (*Macrophomina phaseolina*) and leaf crinkle viral disease (Jaimini, 2023). Cercospora leaf spot disease was first reported by Ellis and Martin in 1882 from United States of America on *Vigna catjang*. They named the organism as *Cercospora canescens*. CLS is a major foliar disease of mothbean caused by *Cercospora canescens.* In India Munjal *et al.*, 1962, first reported in Delhi. The disease occurres in all parts of the humid tropical areas of Asia and many other countries (Pandey *et al.*, 2009). The yield decrease due to the Cercospora leaf spot depends upon how early plant are infected and disease starts appearing about 30 to 40 day after sowing. Depending upon the favourable condition of environment of pathogen (Poehlman, 1978). The disease affects the foliar part of plant and in severe form it causes yield loss up to 50-70% (Kumar *et al.*, 2023). Chupp (1953) recorded *Cercospora canescens* on green gram. The causal entity belongs to the class - *Deuteromycetes*, order - *Moniliales* and family- *Dematiaceae*. The fungus causes distinct spots to appear on leaves. These spotsare first brown and later change to grey or filthy grey with a narrow reddish brown edge that has fructifications on both surfaces. The spot's diameter might range from 5 to 10mm. Anon-host-specific, photo-activated toxin called cercosporin is produced by *Cercospora canescens* (Lynch and Geoghegan, 1977). Cercospora leaf spot of mothbean is a very serious disease in Northern-Western region of India, due to huge economicallosses caused by leafs pot pathogen. Efficacy of various fungicides against *Cercospora canescens* has been reported by many workers (Raj *et al.*, 2011, Chanda *et al.*, 2018 and Kumar *et al.*, 2022), but leaf spotd is ease has not been controlled consistently and economically due to prolongedviability and unpredictable nature of fungal propagules (Mishra, 2002 and Chand *et al.*, 2013). Now, it has become an emerging threat of mothbean production and productivity as very minute information is available for the managing of cercospor a leaf spot with newer chemicals. Currently, there are so many combination of fungicides belongs to different groups are available which can be use deffectively for management of cercospora leaf spot over other methods but those cause severe hazards to human health and environment, so alternative eco-friendly approach esto control leafs pot of moth bean is essential. Tominimize these negative impacts, evaluate the effectiveness of phyto extract in cercos pora leafs pot management is needed.

Materials and Methods

Evaluation of fungicides

Efficacy of various fungicides were tested against *Cercospora* leaf spot of moth bean by "Poisoned Food Technique" under *in vitro* conditions. Three different concentrations *viz.*, 100, 250 and 500 ppm of each fungicide were tested. A desired quantity of each fungicide was added separately in sterilized PDA medium and mixed thoroughly under aseptic conditions. A quantity of 20 ml poisoned PDA medium was poured into sterilized Petri plates and allow to solidification. A medium without fungicides were serve as a control. Then after solidify of media each Petri plate will be inoculate with 5 mm mycelium disc of test fungus and incubated at 27±1ºC for 15 days. The experiment was conducted in CRD with three replications. After incubation mycelial growth of the pathogen were recorded and per cent growth inhibition were calculated in each treatment by the formula given by Vincent (1947).

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

Where,

I = Inhibition per cent

C = Colony diameter (mm) in control plate

T = Colony diameter (mm) in treated plate

Evolution of phytoextracts

Bio efficacy of various phytoextracts was tested *in vitro* by "Poisoned Food Technique" against *Cercospora* leaf spot pathogen at 5 &10 per cent concentrations.

Preparation of phytoextracts

Preparation of Phyto-Extrects from each fresh sample of test plant was collected and wash thoroughly in distil water. 100 g of fresh sample was crush by Pestle and Mortar by adding 100 ml sterile distilled water (1:1 w/v). Poison food technique was used to study of anti-fungal activity of plant extract, to get 5 & 10 percent concentration, 5 & 10 ml of stock solution were mixed with 95 & 90 ml of molten PDA. The medium was shaken for uniform mixing of the extract. 20 ml of medium will

Table 1: List of fungicides against *Cercospora canescens* under *in vitro* conditions.

S. No.	Name of fungicides	Concentration (in ppm)		
1	tebuconazole 25.9% EC	100	250	500
2	carbendazim 50 % WP	100	250	500
3	kresoximmethyl 44.3 SC	100	250	500
4	carbendazim 25% WP + mencozeb 50% WP	100	250	500
5	pyraclostrobin 13.3% + epoxyconazole 5% SE	100	250	500
6	azoxystrobin 23% SC + pyraclostrobin 20%WG	100	250	500
7	hexaconazole 4% + zineb 68% WP	100	250	500
8	Control			

be pour into Petri plates. Plate were inoculated with 5 mm mycelial discs taken from the periphery of fungal culture and incubated at 27 ± 1 ^{0}C till the growth of colony touch the periphery in the control plate. The experiment was conducted in CRD with three replication and suitable plates were maintained as control without plant extract.

Evaluations of bio agents

The efficacy of various bio agents was test by Dual Culture Technique against *Cercospora* leaf spot pathogen under *in vitro* conditions**.** The fungus was cultured on PDA. 20 ml of sterilized cooled PDA to be poured into sterilize Petri plate and allowed to solidify. For evaluation of fungal bio control agents mycelial disc of pathogen and bio agent (antagonistic fungus) were placed separately at equal distance on the periphery of the Petri plate containing media. The bacteria were streak at the middle of the Petri plate and mycelial disc of the test fungus were placed on either side in the center of each half of the plate. The plates were incubated at 27 ± 1ºC and zone of inhibition were recorded by calculated the perfect distance between the test fungus and antagonistic organism. The colony diameter of the pathogen in control plate was also recorded. The experiment was conducted in CRD with four replications.

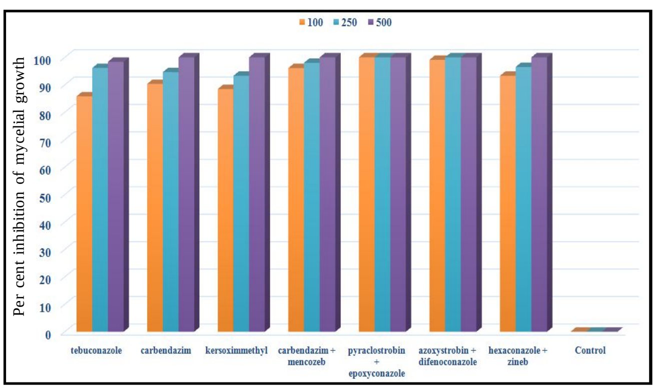

Fig. 1: Efficacy of fungicides against mycelial growth of *Cercospora canescens* at different concentrations *in vitro* conditions.

Results

Evaluation of fungicides, phytoextracts and bio-agents against *Cercospora canescens* under *in vitro* conditions

Evaluation of fungicides against *Cercospora canescens*

Effect of seven contact, systemic and combination fungicides were evaluated along with control at different concentrations *viz.*, 100, 250 and 500 ppm through poisoned food techniques against *Cercospora canescens*. Observations on average colony diameter and per cent inhibition of linear growth over control are presented in (Table 1, Fig. 1 and Plate 1). The results revealed that all the nine fungicides evaluated were significantly reduced the mycelial growth of *C. canescens* over control but their concentration were different to each other. Among these fungicides, the combination fungicide pyraclostrobin 13.3% + epoxyconazole 5% was found more effective

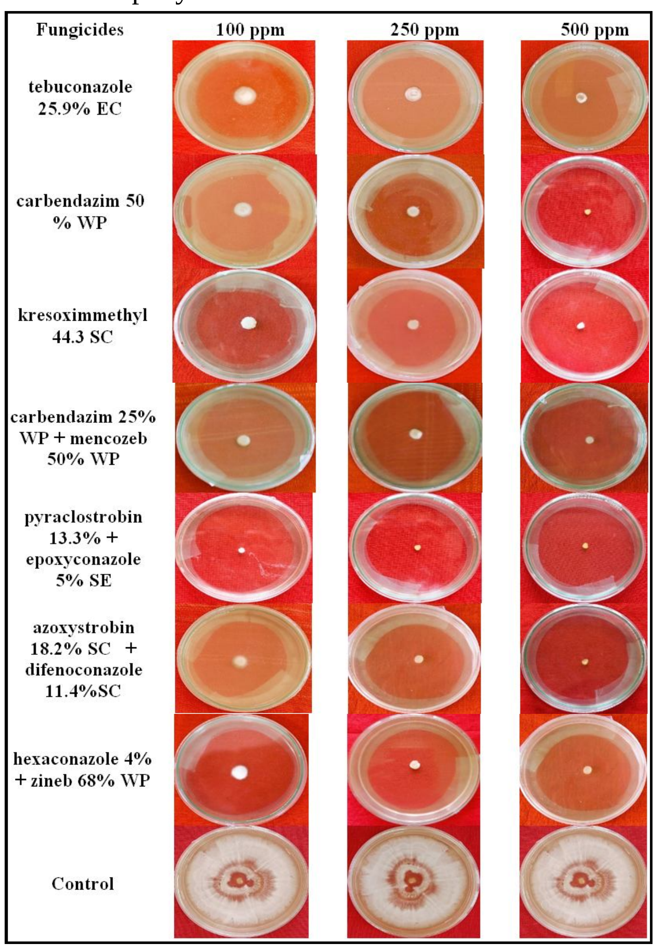

Plate 1: Evaluation of fungicides against *Cercospora canescens* at different concentrations under *in vitro* conditions.

Table 2: List of Phyto-extracts against *Cercospora canescens* under *in vitro* conditions.

S. No.	Common Name	Scientific name	Part used	Con. in (%)	
1	Garlic	*Allium sativum*	Cloves	5	10
2	Ginger	*Zingiber officinale*	Rhizome	5	10
3	Tulsi	*Ocimum sanctum*	Leaves	5	10
4	Aak	*Calotropis gigantean*	Leaves	5	10
5	Datura	*Datura stramonium*	Leaves	5	10
6	Neem	*Azadirachta indica*	Leaves	5	10
7	Control				

Con.: Concentration

and inhibited 100% mycelial growth of *C. canescens* at 100, 250 and 500 ppm concentrations followed by azoxystrobin 18.2% SC + difenoconazole 11.4% SC (99.08%, 100%, 100%) respectively over control. Carbendazim 25% WP + mencozeb 50% WP inhibit (96.07%) and (98.01%) at 100 and 250 ppm concentrations and complete mycelial growth inhibition (100%) was observed at 500 ppm concentration. hexaconazole 4% + zineb 68% WP inhibit (93.28%) and (96.50%) at 100 and 250 ppm concentrations and complete mycelial growth inhibition (100%) was observed at 500 ppm concentration. Similarly, the next fungicides carbendazim 50 % WP was also found effective and inhibited the growth of *C. canescens* (90.32%, 94.62%, 100%) followed by kersoximmethyl 44.3 SC (88.44%, 93.28%, 100%) respectively at 100, 250, 500 ppm concentrations. Tebuconazole 25.9% EC was found less effective with inhibition of mycelial growth by 85.80, 96.13 and 98.39 per cent at 100. 250 and 500 ppm concentrations, respectively over control.

Evaluation of phytoextracts against *Cercospora canescens*

The effect of six phytoextracts were evaluated along with control at two concentrations 5% and 10% through poisoned food techniques against *Cercospora canescens* pathogen. Out of all treatments *Allium sativum* showed significantly higher mycelial growth inhibition over control (Table 2, Fig. 2 & Plate 2).

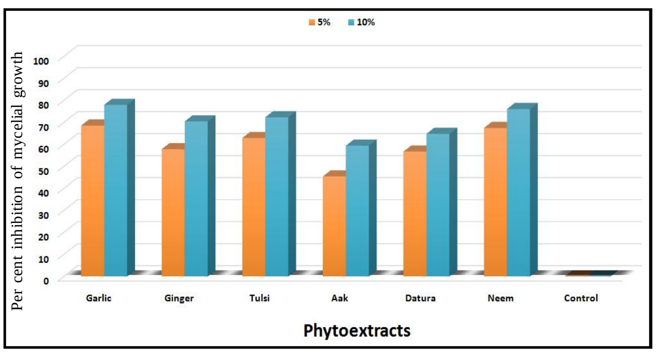

Fig. 2: Efficacy of phytoextracts against mycelial growth of *Cercospora canescens* at different concentrations *in vitro* conditions.

Amongst the six plant extracts evaluated, garlic extract at 10 and 5 per cent concentrations was found best in inhibiting the mycelial growth of *C. canescens* (77.96% and 68.55%) and found significantly superior over all the other extracts, followed by *Azadirachta indica* leaf extract (76.07% and 67.47%), *Ocimum sanctum* leaf extract (72.32% and 62.90%), *Zingiber officinale* extract (70.43% and 57.79%) and *Datura stramonium* leaf extract (64.78% and 56.72%). While, *Calotropis giganntea* leaf extract was found least effective with inhibition of mycelial growth (59.40% and 45.43 %) at 10 and 5 per cent concentrations respectively over control.

Evaluation of bio agents against *Cercospora canescens*

The effect of four bio agents was evaluated along with control through Dual culture techniques against *Cercospora canescens* pathogen. Out of all treatments

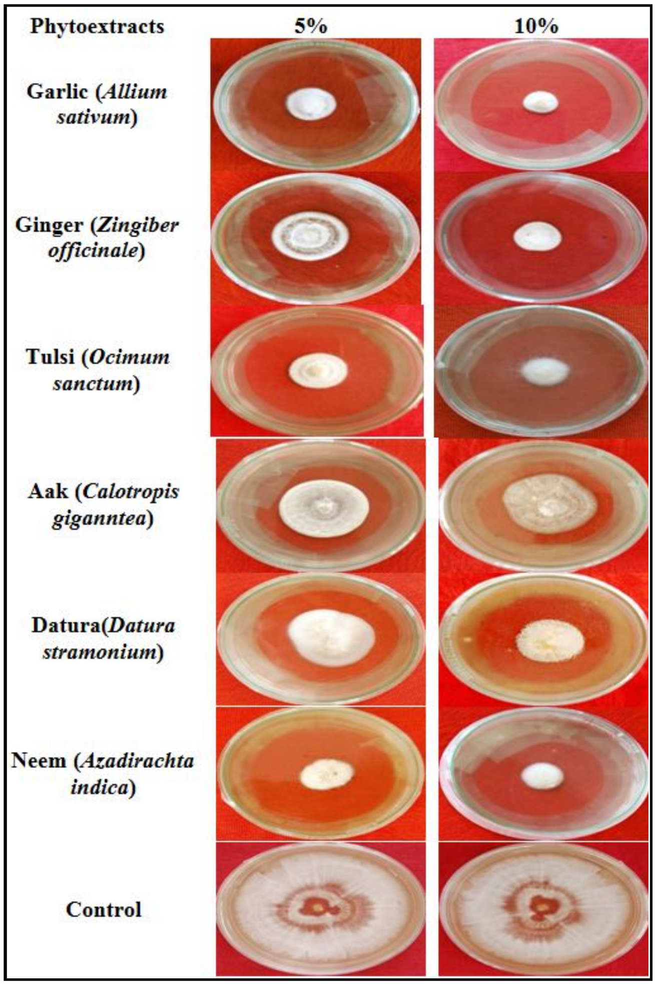

Plate 2: Evaluation of phyto-extracts against *Cercosporacanescens* at different concentrations under *in vitro* conditions.

Table 3: List of bio agents against *Cercospora canescens* under *in vitro* conditions.

S. No.	Name of bio agents
1	*Trichoderma viride*
2	*Trichoderma harzianum*
3	*Pseudomonas fluorescens*
4	*Bacillus subtilis*
5	Control

Trichoderma harzianum showed significantly higher mycelial growth inhibition over control (Table 3, Fig. 3 & Plate 3).

Among all the bio agents tested, *Trichoderma harzianum* (78.83%) and *Trichoderma viride* (74.59%) were found to be significantly superior in inhibiting the mycelial growth of *C. canescens* followed by *Bacillus subtilis* (68.95%). While, least inhibition of mycelial growth of *C. canescens* was recorded in *Pseudomonas fluorescens* (63.50%).

Discussion

Importance of chemicals cannot be denied to management of plant diseases. Seven fungicides were evaluated *in vitro* condition by poisoned food techniques against *Cercospora canescens* inciting cercospora leaf spot of mothbean. All the tested fungicides showed significantly higher mycelial growth inhibition over control. Among them pyraclostrobin 13.3% + epoxyconazole 5% gave complete mycelial growth inhibition (100%) of at 100, 250 and 500 ppm concentrations, respectively. Second best of azoxystrobin 18.2% SC + difenoconazole 11.4% SC then carbendazim 25% WP + mencozeb 50% WP, hexaconazole 4% + zineb 68% WP, carbendazim 50 % WP, kersoximmethyl 44.3 SC inhibit complete mycelial growth inhibition (100%) was observed at 500 ppm concentration. While, tebuconazole 25.9% EC was found less effective with inhibition of mycelial growth by 85.80, 96.13 and 98.39 per cent at 100. 250 and 500 ppm concentrations, respectively over control. These similar results obtained by Kavyashree *et al.*, (2017) under laboratory conditions, evaluation of different fungicides against *Cercospora canescens* of greengram revealed that hexaconazole 5 EC (0.1%), carbendazim (25%) + mancozeb (50%) WS (0.05%) or mancozeb 75 WP (0.1%) and propiconazole 25 EC and trifloxystrobin (25%) + tebuconazole (50%) w/w (0.05%) resulted into 100 per cent inhibition of mycelial growth. Another similar finding was also observed by Raghuvanshi *et al.*, (2022) and they evaluated efficacy of different fungicides and found carbendazim was highly effective in mycileal growth inhibition at 100 ppm concentration against *Cercospora canescens*. Kumar *et al.*, (2020) studied seven fungicides and found Hexaconazole, Hexaconazole + Captan and Carbendazim + Mancozeb were inhibit 100 per cent mycelial of *C. canescens*. Kumar *et al.*, (2022) evaluated nine fungicides and found trifloxystrobin 25% + tabuconazole 50% WG and carbendazim 12% + mancozeb 63% WP were highly effective at 200 ppm concentration.

In vitro efficacy of six botanicals were tested that some of the botanicals reduced the cercospora leaf spot disease intensity as compared to untreated control. *Allium sativum* extract was found to be best in inhibiting the mycelial growth (77.96%) of *Cercospora canescens* and found significantly superior over all the other extracts, followed by neem leaf extract (76.07%), tulsi leaf extract (72.32%), ginger extract (70.43%) and datura leaf extract (64.78%). Aak leaf extract was found least effective with inhibition of mycelial growth (59.40%) at 10 per cent concentration. The fungicidal spectrum of Garlic has been reported by Singh *et al.*, (2014) observed that the maximum percentage inhibition of mycelial growth was obtained from Garlic at 10.0 and 15.0 per cent, followed by Datura and Neem leaf extract against *Cercospora canescens.* Venturoso *et al.*, (2011) found Garlic clove extract was a good inhibitor of *C. Kikuchii* and also by Poornima *et al.*, (2011) on *Cercospora beticola*. Sheshma and Kumar (2017) evaluated phytoextrects against *Cercospora canescens* and observed the extract of neem leaf was highly effective

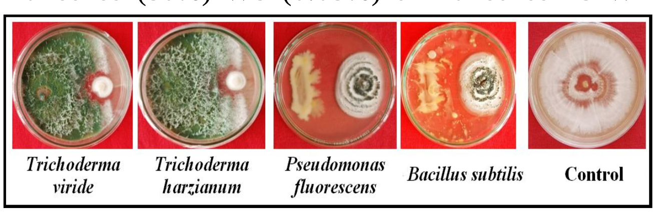

Plate 3: Efficacy of bio agents against mycelial growth of Cercospora canescens at differentconcentrations *in vitro* conditions.

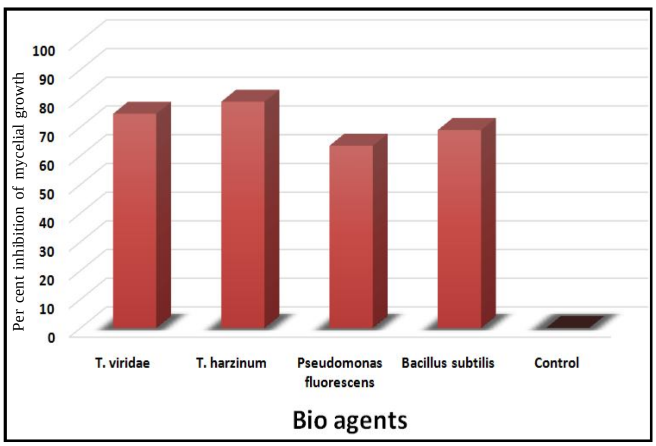

Fig. 3: Efficacy of bio agents against mycelial growth of *Cercospora canescens* at different concentrations *in vitro* conditions.

Table 4: Efficacy of fungicides against *C. canescens* under *in vitro* conditions.

S. No.	Treatment	Per cent inhibition of mycelial growth at different concentration (ppm)*			Mean
		100	250	500	
1	tebuconazole 25.9% EC	85.80(67.86)	96.13(78.65)	98.39(82.70)	93.44
2	carbendazim 50 % WP	90.32(71.88)	94.62(76.59)	100.00(90.00)	94.98
3	kresoximmethyl 44.3 SC	88.44(70.12)	93.28(74.97)	100.00(90.00)	93.90
4	carbendazim 25% WP + mencozeb 50% WP	96.07(78.57)	98.01(81.89)	100.00(90.00)	98.03
5	pyraclostrobin 13.3% + epoxyconazole 5% SE	100.00(90.00)	100.00(90.00)	100.00(90.00)	100.00
6	azoxystrobin 18.2% SC + difenoconazole 11.4%SC	99.08(84.51)	100.00(90.00)	100.00(90.00)	99.69
7	hexaconazole 4% + zineb 68% WP	93.28(74.97)	96.50(79.22)	100.00(90.00)	96.59
8	Control	0.00	0.00	0.00	
	Mean	81.62	96.93	87.30	
Factors		**SEm±**		**CD (p=0.05)**	
Factor (A)		**0.052**		**0.147**	
Factor (B)		**0.084**		**0.240**	
Factor (A × B)		**0.146**		**0.416**	

*Average of three replications; Figure in parenthesis are angular transformed value

@10% concentration. Kumar *et al.*, (2022) evaluated eight botanicals and found neem seed kernel extract was highly effective in mycelial growth inhibition (58.00%) at 15% concentration followed by tulsi leaf extract (52.96%) and neem leaf extract (44.68%), turmeric extract (27.31%) and giloy extract (25.31%) at 15% concentration. Further, Raghuvansi *et al.*, (2022) evaluated five phyto-extrects against *Cercospora canescens* and found garlic (87.86%) was highly effective against pathogen at 10% concentration followed by tulsi (87.14%), onion (86.90%), ginger (86.43 %) and neem (77.38%).

Table 5: Efficacy of phytoextracts against *C. canescens* under *in vitro* conditions.

S. No.	Treatment	Percent inhibition of mycelial growth at different concentraton		Mean
		5 %	10 %	
1	Garlic (*Allium sativum*)	68.55 (55.88)	77.96 (61.99)	73.25
2	Ginger (*Zingiber officinale*)	57.79 (49.48)	70.43 (57.05)	64.11
3	Tulsi (*Ocimum sanctum*)	62.90 (52.47)	72.32 (58.25)	67.61
4	Aak (*Calotropis giganntea*)	45.43 (42.38)	59.40 (50.42)	52.42
5	Datura (*Datura stramonium*)	56.72 (48.86)	64.78 (53.59)	60.75
6	Neem (*Azadirachta indica*)	67.47 (55.23)	76.07 (60.71)	71.77
7	Control	0.00	0.00	0.00
Factors		**SEm±**	**CD(p=0.05)**	
Factor (A)		**0.151**	**0.440**	
Factor (B)		**0.283**	**0.824**	
Factor (A × B)		**0.400**	**1.165**	

*Average of three replications; Figure in parenthesis are angular transformed value

Besides chemical control, biological method of control is an effective, eco-friendly and alternative approach for any disease management practice. These antagonistic organisms act on the pathogen by different mechanisms *viz.*, competition, lysis, antibiosis, siderophore production and hyper-parasitism (Vidyasekaran, 1999). Among the bioagents applied during present investigation, *Trichoderma harzianum* (78.83%) and *T. viride* (74.60%) were found to be best in inhibiting mycelial growth of *C. canescens* and least per cent inhibition of mycelial growth was observed in *Pseudomonas fluorescens* (63.50%). Present studies recorded significant mycoparasitism of species of *Trichoderma viz. T. harzianum and T. viride* which showed higher

Table 6: Efficacy of bio agents against *C. canescens* under *in vitro* conditions.

S. No.	Treatment	Per cent inhibition of mycelial growth*
1	*Trichoderma viride*	74.59(59.73)*
2	*Trichoderma harzianum*	78.83(62.60)
3	*Pseudomonas fluorescens*	63.50(52.84)
4	*Bacillus subtilis*	68.95(56.13)
5	Control	0.00
SEm±		0.244
CD(p=0.05)		0.743
CV %		0.854

*Average of four replications
Figures in parenthesis are angular transformed value

inhibition of mycelial growth of pathogen compared to bacterial antagonists. It may be due to production of antibiotic substance (viridin) produced by *T. harzianum* as reported by Brain (1951) and also due to the penetration of the antagonistic hyphae into hyphae of pathogen at the place of contact as confirmed by Mukherjee *et al*., (2000). Similar results were reported by Siddaramaiah (1986) in leaf spot of mulberry caused by *C. moricola*. Swamy (2010) evaluated bio agents against *Cercospora capsici* and observed that *Trichoderma viride* inhibit maximum mycelial growth of pathogen. Another similar finding was also observed by Raghuvanshi *et al*., (2022) evaluated antagonistic efficacy of *Trichoderma*spp. against *cercospora canescens* found *Trichoderma viride* (75.71 %)was better in inhibiting the radial growth as compared to *T. harzianum* (54.05 %).

Acknowledgment

The work has been undertaken as part of the of M.Sc. research program at Instructional farm, College of Agriculture Jodhpur, Agriculture University, Jodhpur. I thankful to the Advisory committee for their support during master degree program.

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