Plant Archives Vol. 25, No. 2, 2025 pp. 1973-1979 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

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

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.2.286

EFFICACY OF BIO-AGENTS AND BOTANICALS AGAINST CERCOSPORA LEAF SPOT IN OKRA (*ABELMOSCHUS ESCULENTUS* L.) CAUSED BY *CERCOSPORA MALAYENSIS* (STEV. AND SOLH.)

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(Date of Receiving-18-06-2025; Date of Acceptance-01-09-2025)

ABSTRACT

Okra is a key vegetable grown during the *Kharif* season in India. However, its cultivation faces major challenges due to Cercospora leaf spot — a serious disease caused by the fungus *Cercospora malayensis* (Stev. and Solh.). This infection can significantly reduce crop yield and quality. The limited effectiveness and environmental concerns associated with chemical fungicides have driven the search for eco-friendly alternatives to manage this disease. A field study was conducted during the *Kharif* season of 2023 to evaluate the efficacy of bio-agents and botanicals against Cercospora leaf spot in okra. The experiment was carried out under *in-vivo* conditions. Among the treatments tested, T_6- *Trichoderma viride* (Seed Treatment) + mancozeb 75% WP Foliar Spray @ 2.5% + 0.1%) consistently showed the most promising results. This treatment significantly reduced disease intensity at 50, 70 and 90 days after sowing, recording values of 7.10%, 13.43%, and 21.90%, respectively. In addition to effective disease suppression, T_6 also promoted better plant growth. The maximum plant height was observed in this treatment at 30, 60 and 90 days after sowing, reaching 20.62 cm, 41.07 cm, and 78.40 cm, respectively. Most notably, the yield was significantly improved under T_6, with an increase to 9.12 tonnes per hectare, compared to the untreated control (T_0), confirming its superior performance in both disease management and crop productivity.

Key words : *Abelmoschus esculentus*, Bio-agents, Botanicals and *Cercospora malayensis*, *Trichoderma viride*.

Introduction

Okra (*Abelmoschus esculentus* L.) is an important and popular vegetable crop in tropical and sub- tropical regions. It is a tender plant, grows well in hot weather, resulting in early emergencies and higher yields than in cool season. In Egypt, there is different production seasons that due to the mild climatic conditions. Okra is mainly considered as a summer crop. The fresh fruit is a good source of vitamins, minerals and plant protein (Moula *et al.*, 2018).

The crop requires a long, warm and humid growing period for its growth and development and sensitive to frost and extremely low temperature. A temperature range between 24-28°C is normal and temperature beyond 40-42°C, flowers may desiccate and drop causing yield loss. Well drained rich soils having pH range 6.0-6.8 is ideally suited for crop growth and development (Raghuvanshi *et al.*, 2022).

India ranks first in world for okra production and commercially grown in Gujrat, Maharashtra, Andhra

Pradesh, Uttar Pradesh, Madhya Pradesh, West Bengal, Assam, Rajasthan, Tamil Nadu, Haryana, Punjab and Karnataka. In the year 2021–22, okra was cultivated across approximately 553.95 thousand hectares in India, yielding a total production of about 7,252.51 thousand metric tonnes. The average productivity stood at 13.09 tonnes per hectare. In Uttar Pradesh alone, the state contributed around 335.01 thousand metric tonnes to the national output (National Horticulture Board, 2021–22).

Okra is susceptible to a variety of common diseases that can severely affect its growth and yield. These include Cercospora leaf spot (*Cercospora abelmoschi*), damping-off (caused by *Pythium* and *Rhizoctonia* species), powdery mildew (*Oidium* species), southern blight (*Sclerotium rolfsii*), verticillium wilt (*Verticillium albo-atrum*), wet rot (*Choanephora cucurbitarum*), alternaria leaf spot, and okra leaf curl virus (Farrag, 2011).

The disease first appears as faint, olive-colored specks on the underside of the leaves. As it progresses, a light brown to grey, mould-like fungal growth gradually spreads to cover the entire lower leaf surface. The infected leaves ultimately dry and defoliate. The disease progress upward from lower leaves and infects stem and fruits and produces similar symptoms. The species belonging to cercospora genus are distributed worldwide and cause wide Cercospora leaf spot (CLS) on most of the plant families (Crous and Braun, 2003). CLS is an Important disease of okra (Amadi, 1994), Cercospora Leaf Spot (CLS) is primarily managed through foliar spray applications (Jacobsen *et al*., 2000). Additional effective practices include deep ploughing, proper disposal or burying of infected plant debris, cultivating resistant varieties, and adopting a crop rotation system of 2–3 years with non-host crops (Ruppel, 1986).

Fungal diseases constitute a major limiting factor to the growth and yield of okra. Okra is affected by several common diseases, including Cercospora leaf spot, damping-off, powdery mildew, leaf blight and fruit rot. Among these, Cercospora leaf spot is considered one of the most serious and damaging diseases. It limits the photosynthetic potential of the leaves, thereby reducing the overall yield performance of the crop (Dania and Sam, 2020).

Materials and Methods

Experimental site

The present investigation was carried out in the Central Research Farm, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology And Sciences (SHUATS), Prayagraj.

Isolation of test fungus

Using a sterile needle, a small portion of the infected stem was taken and placed on a sterile glass slide. Stained using lactophenol and cotton blue and cover it with the coverslip. Then, the microscope used for the examination of morphological characteristics of fungal structures.

Symptom of leaf spot of okra

The species of Cercospora attack various plants such as cow pea, maize, okra, hibiscus, coffee, sweet pea, beet root, palak etc. Two species, *Cercospora malayensis* and *Cercospora abelmoschi* attack all the plant parts. These fungi are air-borne and soil inhabiting, causing premature withering of leaves leads to low yield. Spore and mycelium in diseased plant debris also serve as means of perennation (Sridharan and Rangaswamy, 1968).

Experimental details

Shiv ganga variety were chosen for the experiment. All the package of practices were followed as per the general agronomic practices.

Field preparation

The chosen field was thoroughly prepared according to the layout plan. It was ploughed, cleared of debris, and the soil was finely tilled. After preparation, the entire area was divided into smaller sub-plots for the experiment.

Application of fungicide

Seeds were treated with the slurry of commercially available systemic fungicide Mancozeb 0.2% . It is a systemic fungicide. The slurry was prepared and used for treatment with seeds and allowed the seeds to dry under shade. The treated seeds were sown in the field.

Application of Bio-agents and Botanicals

Trichoderma viride as seed treatment 5% and *Pseudomonas fluorescens* and botanicals as foliar spray 5% conc. were used during experiment

Management of disease

Bio-agents and botanicals were tested in the field to assess their effectiveness. Field experiment was laid out in Randomized Block Design with three replications, during the *Kharif* season of 2023 and 3 sprays were given at an interval of 15 days. Treatments were imposed after appearance of the first disease symptoms and yield Data were obtained after the harvest on physiological maturity.

Observations recorded

Disease intensity : Disease intensity (%) was recorded at 50, 70 and 90 days after incidence Cercospora

Table 1 : Detail of treatment.

Treatments Number	Treatments
T_0	Control
T_1	*Trichoderma viride* S.T. @ 5%
T_2	*Pseudomonas fluorescens*–F.S. @ 5%
T_3	Clove extract – F.S. @ 5%
T_4	Neem oil – F.S. @ 5%
T_5	Eucalyptus Oil – F.S. @5%
T_6	*Trichoderma viride* - S.T. @ 5% + mancozeb 75 % wp + F.S @ 0.2%
T_7	Mancozeb – F.S.@ 0.2%

S.T.= Seed Treatment F.S.=Foliar Spray

leaf spot of (*Cercospora malayensis*) okra.

Disease intensity (%) is calculated by using the following formula was given by Wheeler (1969).

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

Table 2 : Disease intensity scale (Kapadiya and Dhruj, 1999).

Grade	Leaf area covered
0	No symptoms of disease on leaves
1	Small spots covering 1% or less leaf area.
3	Spots small (up to 5 mm in size) covering 1-10% of leaf area
5	Spots enlarging covering 11-25% of leaf area.
7	Spots coalesce to form big patches covering 26-50% of leaf area.
9	Big spots covering 51% or more of leaf area

0 1 3 5 7 9

Plant height (cm) : Plant height was recorded from five randomly selected plants in each plot at 30, 60 and 90 days after sowing. Height was measured from the base of the plant to the tip of the main shoot and expressed in centimeters.

Yield (t/ha) : The yield was recorded from the field

Cost benefit ratio : Benefit cost ratio is the ratio of gross return to cost of cultivation, which can also be expressed as return per rupee invested. This index provides an estimate of the benefit a farmer derives from the expenditure he incurs in adopting a particular cropping system. Any value above 2.0 is considered safe as the farmer gets ¹ .2 for every rupee invested. The benefit cost ratio was calculated using the formula (Reddy and Reddy, 2004).

$$\text{CBR} = \frac{\text{Gross Return (` /ha)}}{\text{Total cost of cultivation (` /ha)}}$$

Results and Discussion

Effect of treatments on disease intensity (%) at 50, 70 and 90 days after sowing

Effect of different bioagents and botanicals were evaluated against *Cercospora malayensis* under field condition for per cent disease intensity at 50, 70 and 90 days after sowing. Showed in Table 3 and Fig. 1.

At 50 days after sowing among all the treatments, minimum per cent disease intensity (%)was recorded with *Trichoderma viride* + mancozeb 75% WP (7.10%) followed by *Trichoderma viride* (9.26%), *Pseudomonas fluorescens* (10.16%), clove extract (11.06%), neem oil (11.97%), eucalyptus oil (12.20%) and maximum per cent disease intensity was recorded in control (14.39%).

Each treatment differed significantly from the control (T_0) in terms of effectiveness. Among the treatments comparing with C.D. value (0.388), (T_1, T_2, T_3 and T_6) were significant to each other however (T_4 and T_5) were found not significant to each other.

At 70 days after swoing among all the treatments, minimum per cent disease intensity (%) was recorded with *Trichoderma viride* + mancozeb 75% WP (13.43%) followed by *Trichoderma viride* (15.40%), *Pseudomonas fluorescens* (16.72%), clove extract (17.42 %), neem oil (18.19%), eucalyptus oil (18.56 %) and maximum per cent disease intensity was recorded in control (22.32%).

Each treatment differed significantly from the control (T_0) in terms of effectiveness. Among the treatments

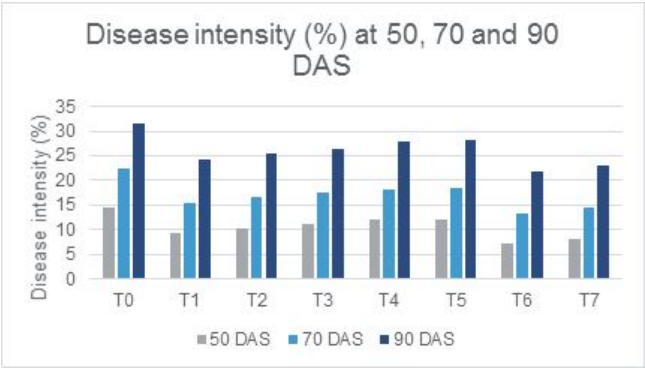

Fig. 1 : Effect of treatments on the disease intensity (%).

Table 3 : Effect of treatments on per cent disease intensity of okra at 50, 70 and 90 days after sowing.

Treatments No.	Treatment details	50 DAS	70 DAS	90 DAS
T_0	Control	14.39	22.32	31.45
T_1	*Trichoderma viride* – S.T. @5%	9.26	15.40	24.36
T_2	*Pseudomonas fluorescens*–F.S. @5%	10.16	16.72	25.40
T_3	Clove extract – F.S. @5%	11.06	17.42	26.30
T_4	Neem oil – F.S. @5%	11.97[a]	18.19[a]	27.75[a]
T_5	Eucalyptus Oil – F.S. @5%	12.20[a]	18.56[a]	28.20[a]
T_6	*Trichoderma viride* S.T. @5%+ mancozeb 75 % wp F.S. @ 0.2 %	7.10	13.43	21.90
T_7	Mancozeb – F.S.@0.2%	8.24	14.50	22.89
	CD (5%)	0.388	0.554	0.539
	S. Ed±	0.18	0.26	0.25

*Average of three replications
*Data followed by same alphabets in a column are non-significant to each other at 5% level
S.T- Seed Treatment; F.S- Foliar Spray; DAS- Days After Sowing

Table 4 : Effect of treatments on plant height (cm) of okra at 50, 70 and 90 DAS.

Treatments No.	Treatment details	50 DAS	70 DAS	90 DAS
T_0	Control	10.36	29.27	68.36
T_1	*Trichoderma viride* – S.T. @ 5%	17.44	37.28	75.35
T_2	*Pseudomonas fluorescens*–F.S. @ 5%	16.54	35.65	74.52
T_3	Clove extract – F.S. @5%	13.70[a]	33.42[a]	73.55[a]
T_4	Neem oil – F.S. @5%	13.40[ab]	33.20[ab]	73.20[ab]
T_5	Eucalyptus Oil – F.S. @5%	12.90[b]	32.92[b]	72.81[b]
T_6	*Trichoderma viride* S.T. @5%+ mancozeb 75 % wp F.S. @ 0.2 %	20.62	41.07	78.40
T_7	Mancozeb – F.S.@0.2%	14.26	34.54	74.09
	CD (5%)	0.695	0.408	0.552
	S. Ed±	0.23	0.16	0.19

*Average of three replications
*Data followed by same alphabets in a column are non-significant to each other at 5% level
S.T- Seed Treatment; F.S- Foliar Spray; DAS- Days After Sowing

comparing with C.D. value (0.554), (T_1, T_2, T_3 and T_6) were significant to each other however (T_4 and T_5) were found not significant to each other.

At 90 days after sowing among all the treatments, minimum per cent disease intensity (%) was recorded with *Trichoderma viride* + mancozeb 75% wp (21.90%) followed by *Trichoderma viride* (24.36%), *Pseudomonas fluorescens* (25.40%), clove extract (26.30%), neem oil (27.75%), eucalyptus oil (28.20%) and maximum per cent disease intensity was recorded in control (31.45%).

Each treatment differed significantly from the control (T_0) in terms of effectiveness. Among the treatments comparing with C.D. value (0.539), (T_1, T_2, T_3 and T_6) were significant to each other however (T_4 and T_5) were found not significant to each other.

In the present studies minimum disease intensity in at 50, 70 and 90 days after sowing was recorded with *Trichoderma viride* + mancozeb 75% wp followed by *Trichoderma viride* and found effective over other treatment. The probable reason for such finding may be due to the inhibitory impact of bio-agents because of hyper parasitism /mycoparasitism, opposition for area and dietary supply and opposed chemical produced with the aid of using them. *Trichoderma viride* which can reduce the severity of plant diseases by inhibiting plant pathogens in the soil through their highly potent antagonistic and mycoparasitic activity and mancozeb 75% WP is a broad-spectrum fungicide with protective action. The product exhibits fungitoxic properties upon exposure to air, where it converts into an isothiocyanate compound that inactivates the sulphahydryl (–SH) groups in fungal

enzymes, thereby inhibiting their activity. These observations align with the findings of Gurjar *et al.* (2003), Khan and Gangopadhyay (2008), Dataram *et al.* (2016), Reddy *et al.* (2021) and Sarangi *et al.* (2023). In particular, studies by Reddy *et al.* (2021) and Sarangi *et al.* (2023) reported that the combination of *Trichoderma viride* and mancozeb 75% WP was the most effective treatment, resulting in the lowest disease intensity compared to all other treatments.

Effect of treatments on plant height (cm) of okra

Effect of different bioagent and botanical were evaluated against *Cercospora malayensis* under field condition for plant height (cm) at 50, 70 and 90 days after sowing showed in Table 4 and Fig. 2.

At 50 DAS maximum plant height (cm) was recorded with *Trichoderma viride* + mancozeb 75% wp (20.62cm) followed by *Trichoderma viride* (17.44cm), *Pseudomonas fluorescens* (16.54cm), clove extract (13.70cm), neem oil (13.40cm), eucalyptus oil (12.90cm) and minimum plant height (cm) was recorded in control (10.36 cm).

Each treatment differed significantly from the control (T_0) in terms of effectiveness. Among the treatments comparing with C.D. value (0.695), (T_1, T_2 and T_6) were significant to each other however (T_3 and T_4) and (T_4 and T_5) were found not significant to each other.

At 70 DAS maximum plant height (cm) was recorded with *Trichoderma viride* + mancozeb 75% wp (41.07cm) followed by *Trichoderma viride* (37.28cm), *Pseudomonas fluorescens* (35.65cm), clove extract (33.42cm), neem oil (33.20cm), eucalyptus oil (32.92cm) and minimum plant height (cm) was recorded in control (29.27cm).

Table 5 : Effect of treatments on yield (t/ha) of okra.

Treatments No.	Treatment details	Yield (t/ha)	C:B ratio
T_0	Control	5.10	1:1.18
T_1	*Trichoderma viride* – S.T. @5%	6.83	1:1.56
T_2	*Pseudomonas fluorescens*–F.S. @5%	6.36^{b}	1:1.43
T_3	Clove extract – F.S. @5%	6.01^{bc}	1:1.37
T_4	Neem oil – F.S. @5%	5.90^{c}	1:1.34
T_5	Eucalyptus oil – F.S. @5%	5.70^{c}	1:1.30
T_6	*Trichoderma viride* S.T. @5%+ mancozeb 75 % wp F.S @ 0.2 %	9.10^{a}	1:2.08
T_7	Mancozeb – F.S.@0.2%	8.6^{a}	1:1.97
	CD (5%)	0.590	
	S.Ed±	0.20	

*Average of three replications

*Data followed by same alphabets in a column are non-significant to each other at 5% level

S.T- Seed Treatment; F.S- Foliar Spray.

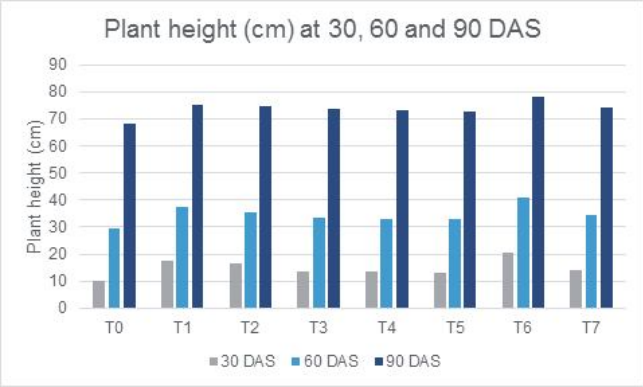

Fig. 2 : Effect of treatments on the plant height (cm).

Fig. 3 : Effect of treatments on yield (t/ha) of okra.

Each treatment differed significantly from the control (T_0) in terms of effectiveness. Among the treatments comparing with C.D. value (0.408), (T_1, T_2 and T_6) were significant to each other however (T_3 and T_4) and (T_4 and T_5) were found not significant to each other.

At 90 DAS maximum plant height (cm) was recorded with *Trichoderma viride* + mancozeb 75 % wp (78.40cm) followed by *Trichoderma viride* (75.35cm), *Pseudomonas fluorescens* (74.52 cm), clove extract (73.55cm), neem oil (73.20cm), eucalyptus oil (72.81cm) and minimum plant height (cm) was recorded in control (68.36cm).

Each treatment differed significantly from the control (T_0) in terms of effectiveness. Among the treatments comparing with C.D. value (0.552), (T_1, T_2 and T_6) were significant to each other however (T_3 and T_4) and (T_4 and T_5) were found not significant to each other.

In the present studies maximum plant height at 50, 70 and 90 DAS was recorded with *Trichoderma*

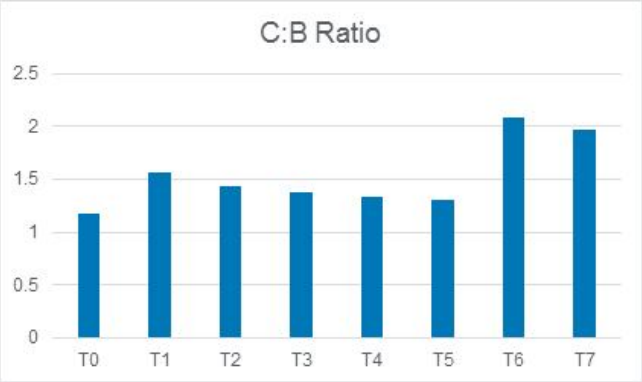

Fig. 4 : C:B ratio.

viride + mancozeb 75% WP followed by *Trichoderma viride* and found effective over other treatment. The probable reason for such finding may be attributed to the soil application of *Trichoderma viride* which might have affected the fungal growth through the production of cell wall degrading enzyme during direct contact and may also worked as plant growth promoting rhizobacteria (PGPR) by producing IAA which may enhance plant growth and vigor supporting increase in plant height. These results were similar to the findings of Devappa and Thejakumar (2016) and Sarangi *et al.* (2023). In the studies conducted by Sarangi *et al.* (2023), maximum plant height was observed with *Trichoderma viride* + mancozeb 75% WP and was significantly effective over all the other treatments.

Effect of treatments on Yield (t/ha) and C:B ratio of okra

Data recorded in Table 5 showed that, among all the treatments, maximum yield (t/ha) and C:B ratio was recorded with T_6- *Trichoderma viride* + mancozeb 75% wp (9.10t/ha and C:B 1:2.08) followed by T_1- *Trichoderma viride* (6.83t/ha and C:B 1:1.56), T_2- *Pseudomonas fluorescens* (6.36t/ha and C:B 1:1.43), T_3-clove extract (6.01t/ha and C:B 1:1.37), T_4-neem oil (5.90t/ha and C:B 1:1.34), T_5-eucalyptus oil (5.73t/ha and C:B ratio 1:1.30) and minimum yield and C:B ratio was recorded in T_0-control (5.1t/ha and C:B 1:1.56).

Each treatment differed significantly from the control (T_0) in terms of effectiveness. Among the treatments comparing with C.D. value (0.590), (T_1 and T_6) were significant to each other however (T_6 and T_7), (T_2 and T_3), (T_3, T_4 and T_5) and (T_4 and T_5) were found not significant to each other.

In the present studies, maximum yield was recorded with *Trichoderma viride* + mancozeb 75% wp followed by *Trichoderma viride* and found effective over other treatments. This result was similar to the finding of Reddy *et al.* (2021), in that the maximum yield was found in *Trichoderma viride* + mancozeb 75% WP (7.96t/ha) and was effective over other treatments.

Conclusion

Based on the result obtained from present investigations, it was found that bio agents and botanicals *Trichoderma viride* S.T. @5%+ mancozeb 75% wp F.S @ 0.2% application was most effective against *Cercospora malayensis* which causes Cercospora leaf spot of okra. Followed by *Trichoderma viride* and *Pseudomonas fluorescens*, therefore it may be recommended for the better management of Cercospora leaf spot of okra. Results of the present study were found to be significantly effective under Prayagraj agro-climatic conditions. It may vary with region and climatic conditions, therefore, for validation of the results mores such trials should be carried out in future.

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