

Estimation of Disease Intensity against *Cercospora* leaf spot of Okra (*Abelmoschus esculentus* L.) Moench using bio-control agents with chemical fungicides under Prayagraj Condition of India.

Comment [Id1]: Please justify why biocontrol agents were used with chemical fungicides or rather the comparison was made in disease reduction

Re-write title of the manuscript

ABSTRACT

Okra (*Abelmoschus esculentus* L.) also known as bhendi is one of the most common vegetable preferred in every household of India. *Cercospora* leaf spot incited by *Cercospora* spp. is one of the emerging diseases in all regions. An experiment was conducted in CRF SHUATS, Prayagraj in Kharif season of 2022 to evaluate the efficacy of bioagents and chemicals viz., T₀ – Untreated control, T₁ Mancozeb (1%) + *Trichoderma*(4%), T₂ - Mancozeb (1%) + *Pseudomonas*(4%), T₃ Mancozeb (1%) + *Bacillus subtilis*(4%), T₄ - Mancozeb (1%) + *Trichoderma*(2%) + *Pseudomonas*(2%), T₅ - Mancozeb (1%) + *Pseudomonas*(2%) + *Bacillus subtilis*(2%), T₆ Mancozeb (1%) + *Bacillus subtilis*(2%) + *Trichoderma*(2%), T₇ - Mancozeb (1%) against *Cercospora* leaf spot of okra. *C. abelmoschi* initiates with sooty black, angular spots and cause heavy defoliation. Studies revealed that minimum disease intensity was observed in T₄ - Mancozeb (1%) + *Trichoderma*(2%) + *Pseudomonas*(2%) and is hereby considered as the best treatment out of all the treatments.

Comment [Id2]: Mention species of *Cercospora* inciting leaf spot in okra

Comment [Id3]: Specify the region also, as the research is location specific

Comment [Id4]: Write the economic importance of *Cercospora* leaf spot in okra and per cent yield losses incurred with recent data

Comment [Id5]: Write treatment code as T₀ (Treatment number as subscript)

Keywords: Mancozeb, *Trichoderma*, *Pseudomonas*, *Bacillus*.

1. INTRODUCTION

Okra (*Abelmoschus esculentus* L.) Moench is one of the most widely known species of the family Malvaceae and an economically important vegetable crop grown in tropical climate of temperature range between 25^o to 35^oc. The name "Okra" derives from one of the Niger-Congo group of languages. "Okra" originated in Ethiopia and was then propagated in North Africa, in the Mediterranean, in Arabia and India by the 12th century BC. "Okra" is known by many local names in different parts of the world. It is called lady's finger in England, gumbo in the United States of America, guino-gombo in Spanish, guibeiro in Portuguese and bhindi in India. (Gemedé et al., 2014).

Okra has nutritional as well as medicinal value. The okra pod is an excellent source of iodine which is necessary for the resistant against throat disease like Goiter. It is good for the people suffering from heart weakness. Some studies

are being developed targeting okra extract as remedy to manage diabetes. Its ripe seeds are roasted, ground and used as a substitute for coffee in some countries. Mature pods and stems containing crude fibre are used in the paper industry. Okra seeds are a potential source of oil, which consists of linoleic acid up to 47.4% and polyunsaturated fatty acid essential for human nutrition. (Singh *et al.*, 2014).

Okra contains Potassium, Sodium, Magnesium and Calcium as principal elements in pods, which contains 17% seeds. Presence of Iron, Zinc, Manganese and Nickel also has been reported (Moyin-Jesu, 2007).

Fresh pods are low in calories (20/100 g), practically no fat, rich in fiber, and with several valuable nutrients. Okra seed is mainly composed of oligomeric catechins (2.5 mg g⁻¹ of seeds), while the mesocarp is mainly composed of hydroxycinnamic (0.2 mg g⁻¹) and quercetin derivatives (0.3 mg g⁻¹). Pods are rich in phenolic compounds with important biological properties like quercetin derivatives, catechin oligomers and hydroxycinnamic derivatives (Arapitsas, 2008).

Okra plant also contains many medicinal properties with it. But before using, it is very necessary to seek advice from a professional. The mucilage can be used as plasma replacement, helpful in washing away toxic substances from the body and have strongly demulcent action (Gemedede *et al.*, 2015).

Among the fungal diseases *Cercospora* leaf spot of bhendi incited by *Cercospora* is one of the most economically important in all regions wherever bhendi is grown. In India, two species of *Cercospora* produce leaf spots on bhendi. *C. malayensis* causes brown, irregular spots and *C. abelmoschi* causes sooty black, angular spots. Both the leaf spots cause severe defoliation and are common during humid seasons. Now a days, this disease incited by *C. abelmoschi* becomes more severe in southern transition zone of Karnataka. Initially the disease symptoms observed on the lower surface of the leaves as in distinct spots in the form of olivaceous specks. Later on, light brown to grey mouldy growth of the fungus covered the entire lower surface. The infected leaves ultimately dry and defoliate. The disease progress upward from lower leaves and infects stem and fruits and produces similar symptoms. (Naik *et al.*, 2017).

Comment [Id6]: Also mention the production, area and productivity of okra in U.P., India and globally

Comment [Id7]: Also mention per cent disease loss of okra due to *Cercospora* leaf spot

Comment [Id8]: Write about the management practices against this particular disease. Focus of the rationale of the study justifying why the present study has used biocontrol agents and also a chemical in management of this particular disease?

2. MATERIAL AND METHODS

The experiment was conducted at the research plot of the Department of Plant Pathology and Central Research Field, Sam Higginbottom University of Agriculture Technology And Sciences, Prayagraj during the *Kharif* season 2022. The selected site was uniform, cultivable with typical sandy loam soil having good drainage.

Table 1. The treatment details.

S. No	Treatments	Treatment Details
1.	T0	Control

Comment [Id9]: Mention field size, plot size, cultivation practices used, seed rate used, variety of seed used, when was pathogen inoculated in the field or it was natural incidence?

Mention treatment design used, how many replications

2.	T1	Mancozeb (1%) + <i>Trichoderma harzianum</i> (4%)
3.	T2	Mancozeb (1%) + <i>Pseudomonas fluorescens</i> (4%)
4.	T3	Mancozeb (1%) + <i>Bacillus subtilis</i> (4%)
5.	T4	Mancozeb (1%) + <i>Trichoderma harzianum</i> (2%) + <i>Pseudomonas fluorescens</i> (2%)
6.	T5	Mancozeb (1%) + <i>Pseudomonas fluorescens</i> (2%) + <i>Bacillus subtilis</i> (2%)
7.	T6	Mancozeb (1%) + <i>Bacillus subtilis</i> (2%) + <i>Trichoderma harzianum</i> (2%)
8.	T7	Mancozeb (1%)

Comment [Id11]: Mention how Mancozeb 1% prepared, which chemical formula used?

Comment [Id10]: Subscript the treatment no. As T₁

Comment [Id12]: Mention how percent of biocontrol agents, for e.g., *Trichoderma* (4%), *Pseudomonas* (4%) was prepared? Is it the biocontrol agents or the formulation used?

Comment [Id13]: Mention trade name of Mancozeb used

Mention from where biocontrol agents procured or it was isolated, describe the process with identifying characteristics

Disease severity scale of *Cercospora* leaf spot

Disease intensity was recorded as grades in five randomly selected plants in each plot at different time that is before spraying, 15 days after the first spray and 15 days after the second spray as per the scale of **Farrag (2011)** which is given below.

Table 2. Disease rating and description

Disease rating /grade	Description
0	No disease
1	Noticeable spotting with some defoliation (< 25%)
3	Spotting heavy with significant defoliation (< 50%)
5	Very heavy leaf spotting with severe defoliation (< 75%)

7	Numerous spots on few remaining leaves and very heavy defoliation (<90%)
9	Very few remaining leaves covered with spots and nearly complete defoliation (<95%)

3.5 Disease intensity (%)

Percentage of Disease intensity will be recorded at 60,75 and 90 days after incidence of *Cercospora* leaf spot. Percentage of Disease intensity will be calculated in accordance with following formula. The disease will be visually assessed in all the plots at weekly interval from first appearance of disease for each treatment. For each plot the number of infected okra plants will be counted and expressed as a percentage of the total number of okra plants in that plot. The mean percentage disease incidence for each treatment will be obtained from the three replications. The data will be further statistically analyzed.

Comment [Id14]: Write about the statistical tool used for analysis

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

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total no. of ratings} \times \text{Maximum disease groups}} \times 100$$

Result and Discussion



Fig 1. Overview of Disease Infested Leaves

Comment [Id15]: Caption should be fully justified and self understandable
e.g., Cercospora disease infected leaves (at what growth stage?)

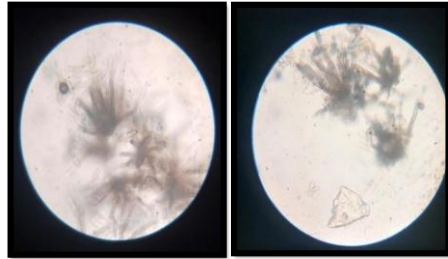


Fig 2. OVERVIEW OF MICROSCOPIC VIEW OF *Cercospora* sp.

Comment [Id16]: Maintain uniformity in using sentence case only
Mention in the caption about at what magnification the microimage was captured, also name the microscope used

Table 3. Effect of treatments on Disease Intensity of *Cercospora* leaf spot of okra at 60, 75 and 90 DAS

Comment [Id17]: Write at footnote of the table, DAS abbreviation

Tr.no	Treatment	DISEASE INTENSITY		
		60DAS	75 DAS	90 DAS
T0	Control	29.183 ^a	36.290 ^a	41.18 ^a
T1	Mancozeb (1%) + <i>Trichoderma harzianum</i> (4%)	21.033 ^d	27.403 ^e	34.44 ^c
T2	Mancozeb (1%) + <i>Pseudomonas fluorescens</i> (4%)	18.810 ^e	25.920 ^f	32.33 ^d
T3	Mancozeb (1%) + <i>Bacillus subtilis</i> (4%)	23.553 ^c	30.810 ^c	36.47 ^b
T4	Mancozeb (1%) + <i>Trichoderma harzianum</i> (2%) + <i>Pseudomonas fluorescens</i> (2%)	14.367 ^g	21.920 ^h	26.66 ^f
T5	Mancozeb (1%) + <i>Pseudomonas fluorescens</i> (2%) + <i>Bacillus subtilis</i> (2%)	15.703 ^f	23.847 ^g	29.33 ^e
T6	Mancozeb (1%) + <i>Bacillus subtilis</i> (2%) + <i>Trichoderma harzianum</i> (2%)	23.847 ^c	28.887 ^d	34.51 ^c
T7	Mancozeb (1%)	27.183 ^b	33.480 ^b	37.33 ^b
C.D (5%)		1.009	1.044	1.867

Comment [Id18]: Maintain uniformity in using sentence case only

Comment [Id19]: Mention the unit of disease intensity

Comment [Id20]: Also mention Sem of the statistical analysis

Comment [Id21]: Mention in footnote of the table: statistical design uses, no. Of replication, CD abbreviation, Sem abbreviation, level of significance, alphabets on the values signify, are values per cent values

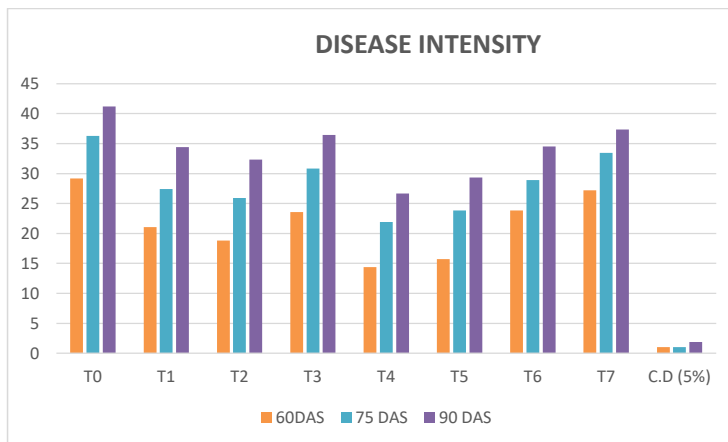


Fig 3. Disease Intensity

Comment [Id22]: Write the caption of the figure, fully justified or self explanatory

Also in the graph, Write axis titles (both X-axis and y-axis), T0 to T7 signifies what?, C.D. value doesn't required to be shown in the graph,

4.1 Disease Intensity:

4.1.1 Disease Intensity at 60 DAS

The data presented in table 3 and depicted in figure 3 reveals that maximum Disease intensity of okra at 60 DAS was recorded in T4 - Mancozeb (1%) + *Trichoderma*(2%) + *Pseudomonas*(2%) (14.36) followed by T5 - Mancozeb (1%) + *Pseudomonas*(2%) + *Bacillus subtilis*(2%) (15.70) and T2 - Mancozeb (1%) + *Pseudomonas*(4%) (18.81) followed by T₁ Mancozeb (1%) + *Trichoderma*(4%) (21.03) , T₆ Mancozeb (1%) + *Bacillus subtilis*(2%) + *Trichoderma*(2%)(23.553), T₃ Mancozeb (1%) + *Bacillus subtilis*(4%) (23.847) as compared to T7 - Mancozeb (1%) (27.18) and T0 – untreated control- (29.18). All the treatments were significant over untreated control. Among the treatments (T₇ and T₄) were statistically non significant to each other

Comment [Id23]: Write unit of each of these values

4.1.2 Disease Intensity at 75 DAS

The data presented in table 3 and depicted in figure 3 reveals that maximum Disease Intensity of okra at 75 DAS was recorded in T4 - Mancozeb (1%) + *Trichoderma*(2%) + *Pseudomonas*(2%) (21.92) followed by T5 - Mancozeb (1%) + *Pseudomonas*(2%) + *Bacillus subtilis*(2%) (23.84) and T2 - Mancozeb (1%) + *Pseudomonas*(4%) (25.92) followed by T₁ Mancozeb (1%) + *Trichoderma*(4%) (27.40) , T₆ Mancozeb (1%) + *Bacillus subtilis*(2%) + *Trichoderma*(2%)(28.88), T₃ Mancozeb (1%) + *Bacillus subtilis*(4%) (30.81) as compared to T7 - Mancozeb (1%) (33.48) and T0 – untreated control- (36.29). All the treatments were significant over untreated control.

Comment [Id24]: Write unit of each of these values

4.1.3 Disease Intensity at 90 DAS

The data presented in table 3 and depicted in figure 3 reveals that maximum Disease Intensity of okra at 90 DAS was recorded in T4 - Mancozeb (1%) + *Trichoderma*(2%) + *Pseudomonas*(2%) (26.66) followed by T5 - Mancozeb (1%) + *Pseudomonas*(2%) + *Bacillus subtilis*(2%) (29.33) and T2 - Mancozeb (1%) + *Pseudomonas*(4%)

Comment [Id25]: Write unit of each of these values

(32.33) followed by T₁ Mancozeb (1%) + *Trichoderma*(4%) (34.44) , T₆ Mancozeb (1%) + *Bacillus subtilis*(2%) + *Trichoderma*(2%)(34.51), T₃ Mancozeb (1%) + *Bacillus subtilis*(4%) (36.47) as compared to T₇ - Mancozeb (1%) (37.33) and T₀ – untreated control- (41.18). All the treatments were significant over untreated control. Among the treatments (T₈ and T₄) , (T₇ and T₂) were statistically non significant to each other.

Conclusion

Based on the ~~observations~~ observations, it can be concluded that the efficacy of combining readily available and ecologically safe bioagents with synthetic safe mancozeb fungicide for the management of *Cercospora* leaf spot of ~~okra~~ okra.

From the critical analysis of the present findings, it can be concluded ~~that after~~ that after the application of all the treatments with three replications, T₄ - Mancozeb (1%) + ~~*Trichoderma*~~ *Trichoderma* (2%) + *Pseudomonas*(2%) is the best treatment as it showed The **Disease Intensity of okra** at 60,75 and 90 DAS which was significantly increased by the use of Mancozeb (1%) + *Trichoderma*(4%) + *Pseudomonas*(4%) under Prayagraj Agro climatic conditions . Based on analysis T₄ - Mancozeb (1%) + ~~*Trichoderma*~~ *Trichoderma* (2%) + ~~*Pseudomonas*~~ *Pseudomonas* (2%) is recommended to control the *cercospora* leaf spot disease in Okra. The present findings were limited to one crop season ~~kharif~~ kharif under the climatic conditions of Prayagraj , U.P. , therefore substantiate the present result more trails are required for further recommendations .

Reference

- Arapitsas (2008)**. Identification and quantification of polyphenolic compounds from okra seeds and skins. *Food Chemistry*, **110**: 1041-1045.
- Faisal, M. and Tiwari, S. (2015)**. Comparison of bio-agents and botanicals with fungicides against tikka and anthracnose diseases of groundnut (*Arachis hypogea* L.). *International Journal of Plant Protection*, **8(1)**: 45-48.
- Gemed, H. F., Ratta, N., Haki, G. D. and Beyene, A. Z. W. F. (2014)**. Nutritional Quality and Health Benefits of Okra (*Abelmoschus esculentus*): A review. *Global Journal of Medical Research*, **14(5)**:2249-4618.
- Kumar, S., Jaiswal, S., Lal, A.A., Kumar, A. and Verma, A. (2017)**. Influenced of natural products and bio-fungicide against tikka disease of groundnut caused by *Cercospora* spp. *The Pharma Innovation Journal*, **6 (3)**:213-216.
- Kumar, R., Meena, A. K., Kumar, V. and Singh, J. (2022)** Isolation of *Cercospora canescens* and management of *Cercospora* Leaf Spot (*Cercospora canescens*) of moth bean through botanicals. *Journal of Experimental Agriculture International*, **44(5)**: 57-63.

Comment [Id26]: Maintain uniformity in the text format

Comment [Id27]: Justify, what should be the application method for using the treatment combination of both biocontrol agents as well as mancozeb

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Naik, G. R. and Jayalakshmi, K. (2017). Evaluation of fungicides against leaf spot of bhendi incited by *Cercospora abelmoschi* under field conditions. *International Journal of Chemical Studies*, **5(5):1210-1212.**

Singh, P., Chauhan, V., Tiwari, B. K., Chauhan, S. S., Simon, S., Bilal, S. and Abidi, A.A. (2014). An overview on okra (*Abelmoschus esculentus*) and its importance as a nutritive vegetable in the world. *International Journal of Pharmacy and Biological Sciences*, **4(2)227- 233.**

DEFINITIONS, ACRONYMS, ABBREVIATIONS

Here is the Definitions section. This is an optional section.

Term: Definition for the term

UNDER PEER REVIEW