



# Comprehensive Disease Management Strategy for Cercospora Leaf Spot in Mungbean: Assessment of Botanicals, Fungicides, and Bio-agents *In vivo*

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## Authors' contributions

This work was carried out in collaboration among all authors. Authors RSR and NS the work was performed by has assist in manuscript drafting. Authors VS and GP has helped in manuscript preparation. All authors read and approved the final manuscript.

## Article Information

DOI: 10.9734/JEAI/2024/v46i52426

## Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/114206>

Original Research Article

Received: 01/02/2024

Accepted: 04/04/2024

Published: 11/04/2024

## ABSTRACT

**Aim:** Worldwide mungbean farming is seriously threatened by the fungal disease *Cercospora canescens*, which causes Cercospora leaf spot and severe yield losses. This study evaluated the effectiveness of fungicides, botanicals, and bioagents *in vivo* with the goal of developing an efficient management approach for Cercospora leaf spot.

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**Study Design:** Randomized block design with ten treatments and three replications.

**Place and Duration of Study:** Department of Plant Pathology, ANDUA&T Kumarganj, Ayodhya (U.P.) India located at latitude 26.47 0N, longitude 82.12 0E and altitude 113m above the sea level during kharif season, 2019.

**Methodology:** The antifungal capabilities of botanical extracts were studied, the pathogen-growth-inhibiting capacity of fungicides was tested, and the potential for bio-agents to suppress the course of disease was analyzed. The effectiveness of plant extracts, including Neem leaf extract, Onion, Garlic clove extract, Tulsi, and Ginger was assessed at a concentration of 10%, against *Cercospora canescens*.

**Results:** Ten per cent concentration was found most effective *in vitro* was further tested *in vivo* at 30 days and 45 days after sowing. Neem leaf extract, Onion, Garlic clove extract, Tulsi, and Ginger @ 10% against *Cercospora canescens*

**Conclusion:** It is very clear from the present studies that the plant extracts which were better in inhibiting the radial growth *In vitro* also showed reduction in disease incidence and per cent disease control was also higher *In vivo* condition.

**Keywords:** *Cercospora canescens*; antifungal; botanical products; mungbean.

## 1. INTRODUCTION

“Pulses are major sources of proteins among the vegetarians in India, and complement the staple cereals in the diets with proteins, essential amino acids, vitamins and minerals. Significant number of nutritional and health benefits are associated with this miracle crop. Among various pulses, Moongbean, scientifically known as *Vigna radiata* and commonly known as mung bean, green gram, or golden gram associated with significant benefits due to its nutritional values and versatility” [1].

Pathogens, including fungal, bacterial, and viral pathogens, pose a substantial threat to mungbean production. Diseases such as powdery mildew, bacterial blight, and viral infections like yellow mosaic virus (MYMV) can cause severe yield losses. In addition, fungal disease *Cercospora* leaf spot caused by *Cercospora canescens*, is a prevalent and economically significant disease affecting mungbean (*Vigna radiata*) cultivation worldwide [2]. This fungal disease results circular to irregular-shaped lesions on leaves transforming from small water-soaked spots in to necrotic lesions. Defoliation and reduced photosynthetic capacity occurs after severe infection resulting significant yield losses. Tropical and subtropical regions where mungbean is extensively cultivated having warm and humid climatic condition favors this disease and hence presents a major barrier to the production of mungbean. Now, talking about its management, integrated disease management is a best way to manage this disease. Cultural practices such as crop rotation, sanitation, and the use of disease-free

seeds can help reduce pathogen inoculum and disease incidence [3]. Additionally, planting resistant or tolerant mungbean varieties offers an effective and environmentally sustainable strategy to mitigate the impact of *Cercospora* leaf spot [2].

Currently we are moving toward zero budget natural farming system and government is also giving special focus to use indigenous technology to manage disease in crop. Sustainable management strategies are required for effectively managing this disease in mungbean. Thus in the present study botanicals (plant leaf extract), bio-agents and fungicide are assessed in the *Cercospora* leaf spot infected mungbean. Current study is focused on investigating the application of different plant extracts including neem, onion, garlic on the *Cercospora* leaf spot infected mungbean. The comparison between fungicide, botanicals and bio-agents application on *Cercospora* leaf spot infected mungbean was analysed. The efficiency of several botanical extracts, fungicide and bio-agent was assessed on *Cercospora* leaf spot under *in vivo* conditions. This research ultimately provide a platform to apply integrated disease management approach for sustainable mungbean production. Hence, farmers may effectively limit the effects of *Cercospora* leaf spot, increase crop output, and guarantee the long-term sustainability of mungbean production systems by integrating multiple control techniques and prioritizing ecological principles.

## 2. MATERIALS AND METHODS

The present investigation were carried out during kharif 2019 at students instructional farm of

**List 1. Detail of treatment**

Treatment	Biological extract	Quantity
T <sub>1</sub>	Neem leaf extract ( <i>Azadirachta indica</i> )	10 ppm/kg seed
T <sub>2</sub>	Garlic bulb extract ( <i>Allium sativum</i> )	10 ppm/kg seed
T <sub>3</sub>	Tulsi extract ( <i>Ocimum sanctum</i> )	10 ppm/kg seed
T <sub>4</sub>	Onion extract ( <i>Allium cepa</i> )	10 ppm/kg seed
T <sub>5</sub>	Ginger extract ( <i>Zingiber officinale</i> )	10 ppm/kg seed
T <sub>6</sub>	<i>Trichoderma viride</i>	4g / kg seed
T <sub>7</sub>	<i>Trichoderma harzianum</i>	4g / kg seed
T <sub>8</sub>	Carbendazim	2g / kg seed
T <sub>9</sub>	Thiram	1g / kg seed
T <sub>10</sub>	Untreated (Check)	

A.N.D. University of Agriculture and Technology Kumarganj, Ayodhya (U.P.) India located at latitude 26.47 ° N, longitude 82.12 ° E and altitude 113 m above the sea level. The experiment was laid out in randomized block design (RBD) with ten treatments and three replications. The plan of layout of the experiment is listed above.

### 2.1 Application of Fungicide Against *Cercospora canescens* in *In Vivo*

Seeds of mungbean variety 'Kopergoan' were moist for 12 hrs. prior to sowing and then treated with the Carbendazim @ 2g/kg seed + Thiram @ 1g/kg seed. Treated seeds of susceptible variety 'Kopergoan' were sown in each plot. *Cercospora* leaf spot infection was recorded at 30 and 45 days after sowing.

### 2.2 Application of Plant Extracts Against *C. canescens* in *In Vivo*

The effective concentration of plant extracts found effective *in vitro* were further tested *in vivo*. After 7 days of sowing, plant extracts (10 per cent) @ 100 ml per kg of soil was thoroughly mixed to determine the effect of plant extract *in vivo*. The experiment was conducted in RBD with 10 treatment including control.

#### 2.2.1 Screening of disease

"The initial onset of the disease, along with disease occurrence and the percentage of disease control, were documented 30 and 45 days post sowing. Percent disease incidence (PDI) and Percent disease control was calculated by using following formula" [4]

Percent disease incidence = (Number of infected plants) / (Total number of plants) × 100

Per cent disease control =  $\frac{C - T}{C} \times 100$

Where,

C = Per cent disease incidence of control plots  
T = Per cent disease incidence in treated plots

### 2.3 Application of Bio-Agents Against *C. canescens* in *In Vivo*

"The pure culture of antagonists was used for seed treatment. Pure culture of *Trichoderma harzianum* and *Trichoderma viride* was prepared with 15 days old culture multiplied in Potato Dextrose Agar and Nutrient agar medium. Potato Dextrose Agar medium and Nutrient agar medium was filled in the Petri plates, approximately up to 1/3 of their total capacity. The medium of each flask were sterilized at 15 p.s.i. for 20 minutes. After sterilization, broth was inoculated with mm disc of actively growing culture of most effective isolate of *Trichoderma harzianum* and *Trichoderma viride*. Flasks were incubated at 25 ± 2°C for 14-15 days till the whole surface was completely covered by mycelial mat. The liquid cultures of the bio-agents were filtered through Whatman filter paper No.44. Mycelial mat was desiccated at room temperature for three days. Dried mycelial mats were grinded by pestle and mortar to get pure powder of these bio-agents" [5].

"This preparation was used for the seed treatment @ of 4 gm/Kg seed of mungbean. The seed was treated with bio-agents separately and sown in plots as described earlier in three replications. The sowing of untreated seeds served as check. Disease screening was recorded at 30 and 45 days after sowing". [4]

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of Plant-Derived Extracts on *Cercospora canescens* Infection on Mungbean

➤ At 30 days after sowing

The minimum disease incidence was found in Garlic (28.45 %), followed by Tulsi (33.77%), Onion (34.45 %), Ginger (34.94 %), Neem (35.55 %). The per cent disease incidence in between however Neem, Tulsi, Onion, and Ginger were significantly at par to each other while minimum disease incidence was found in Garlic (Table 1 and Fig. 1).

The maximum disease reduction was found in Garlic (57.82 %) followed by Tulsi (51.58 %), Onion (50.77 %), Ginger (50.19 %), Neem (49.46 %). The per cent disease reduction in between Neem, Garlic, Tulsi, Onion and Ginger were significantly differed from each other with respect to per cent disease control (Table 1 and Fig. 1).

➤ **At 45 days after sowing**

The minimum disease incidence was obtain in Garlic (31.56 %), followed by Tulsi (35.10%), Onion (36.99 %), Ginger (37.70 %), Neem (39.35%) and control (71.57 %) was recorded at

10 per cent concentration of 45 days after sowing. The per cent disease incidence in Neem, Tulsi, Onion, and Ginger were significantly at par to each other while thus, the minimum disease incidence was observed in Garlic and maximum in Neem.

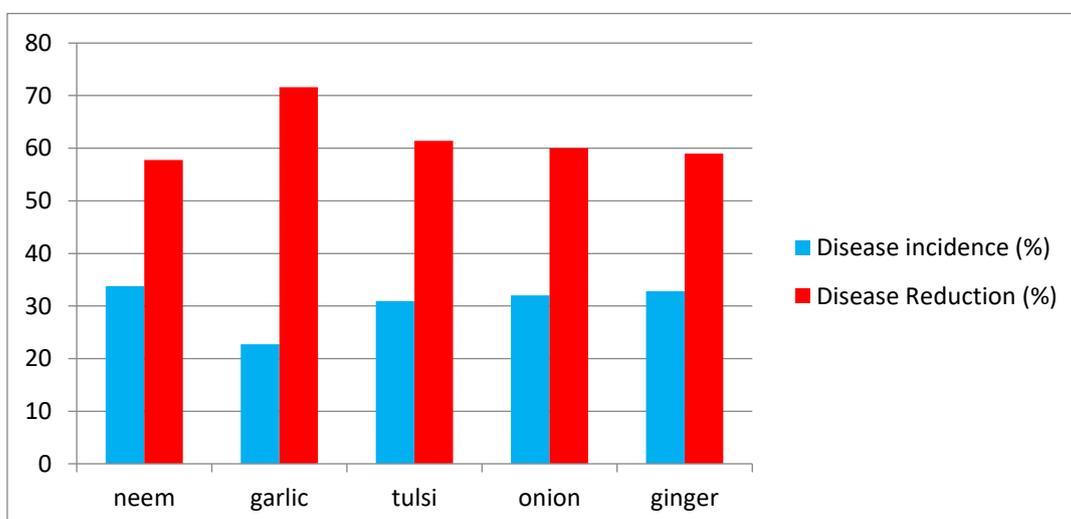
The disease incidence in between Tulsi, Onion and Ginger were at par to each other (Table 2 and fig. 2). Thus, the minimum disease incidence was observed in Garlic and maximum in Neem.

“The maximum disease reduction was obtained in Garlic (56.51 %), followed by Tulsi (52.73 %), Onion (50.64 %) and Ginger (49.86 %), Neem (48.10 %) at 10 per cent concentration of 45 days after sowing. The per cent disease reduction in Neem, Onion and Ginger, Onion and Tulsi, Onion and Tulsi, were at par to each other. Thus, the disease reduction was maximum in Garlic and minimum in Neem was recorded at 30 days and 45 days after sowing”. [6] Per cent disease reduction in rest of the treatment differed significantly (Table 2 and fig. 2).

**Table 1. Effect of plant extracts on disease incidence and disease reduction against *Cercospora canescens* mungbean *In vivo* at 30 days after sowing**

Plant extract	Concentration (%)	Disease incidence (%)	Disease Reduction (%)
Neem (leaf)	10	33.80 (35.55)	57.75 (49.46)
Garlic (bulb)	10	22.70 (28.45)	71.63 (57.82)
Tulsi (leaf)	10	30.90 (33.77)	61.38 (51.58)
Onion (bulb)	10	32.00 (34.45)	60.00 (50.77)
Ginger (rhizome)	10	32.80 (34.94)	59.00 (50.19)
Control	-	80.0 (63.43)	0.00 (0.00)
SEm±	-	1.90	0.63
CD at 5%	-	5.98	0.00

Figure given in parenthesis are transformed value

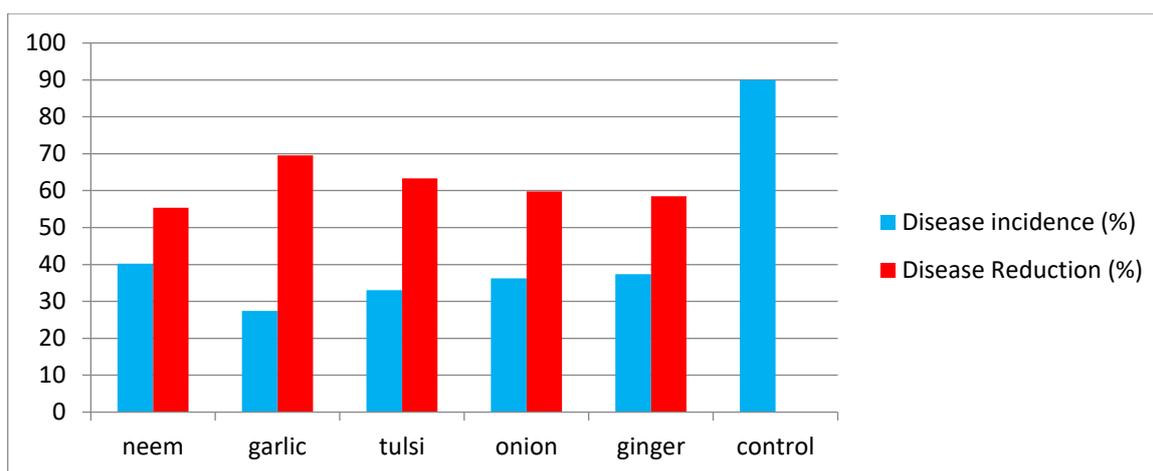


**Fig. 1. Effect of plant extracts on disease incidence and disease reduction against *Cercospora canescens* mungbean *In vivo* at 30 days after sowing**

**Table 2. Effect of plant extracts on disease incidence and disease reduction against *Cercospora canescens* mungbean *In vivo* at 45 days after Sowing**

Plant extract	Concentration (%)	Disease incidence (%)	Disease Reduction (%)
Neem (leaf)	10	40.20 (39.35)	55.33 (48.10)
Garlic (bulb)	10	27.40 (31.56)	69.56 (56.55)
Tulsi (leaf)	10	33.00 (35.10)	63.33 (52.76)
Onion (bulb)	10	36.20 (36.99)	59.78 (50.64)
Ginger (rhizome)	10	37.40 (37.70)	58.44 (49.86)
Control	-	90.00 (71.57)	0.00
SEm±	-	1.42	1.43
CD at 5%	-	4.46	4.50

Figure given in parenthesis are transformed value

**Fig. 2. Effect of plant extracts on disease incidence and disease reduction against *Cercospora canescens* mungbean *In vivo* at 45 days after Sowing**

It is very clear from the present studies that the plant extracts which were better in inhibiting the radial growth *in vitro* also showed reduction in disease incidence and per cent disease control was higher accordingly. *In vivo* condition. Bdliya and Alkali (2010) evaluated “the efficacy of Neem (seed), Garlic (clove), Onion (bulb), Ginger (rhizome) and Pawpaw (leaf extracts) applied as foliar spray in controlling cercospora leaf spot of groundnut in two successive growing seasons. All the plant extracts reduced the incidence and severity of cercospora leaf spot in both seasons compared to the untreated crops. However, Neem (seed) and Garlic (clove) extracts significantly reduced the incidence and severity of the disease compared to the other plant extracts”.

Sheshma and Kumar (2017) evaluated “the efficacy of plant extracts *viz.* Neem leaf extract, Dhatura leaf extract, Garlic clove extract, Arjun leaf extract, Aswagandha leaf extract and Alovera leaf extract @10% against *Cercospora canescens*. *In-situ* (field) experiments were also

carried out in randomized block design with six treatments and three replications. Neem leaf was found to be the most effective treatment and recorded minimum disease intensity (25.69 %), Maximum No of pod per plant, maximum weight of pod (g) and yield (q/ha) followed by Arjun leaf extract, Alovera leaf extract, Aswagandha leaf extract, Dhatura leaf extract and Garlic clove extract”.

### 3.2 Efficacy of Chemicals Against *Cercospora canescens* on Disease Incidence and Disease Reduction *In vivo*

#### ➤ At 30 days after sowing

The minimum disease incidence was found in Carbendazim (10.00 %), followed by Thiram (22.60 %) The per cent disease incidence in between Carbendazim and Thiram were at par to each other and maximum disease incidence was found in Thiram (22.60 %) (Table 3 and Fig. 3).

The maximum disease reduction was found in Carbendazim (87.50%) followed by Thiram (71.75%). The per cent disease incidence in between Carbendazim and Thiram were at par to each other and minimum disease reduction was found in Thiram (71.75%) (Table 3 and Fig. 3).

➤ **At 45 days after sowing**

The minimum disease incidence was found in Carbendazim (14.00 %), followed by Thiram

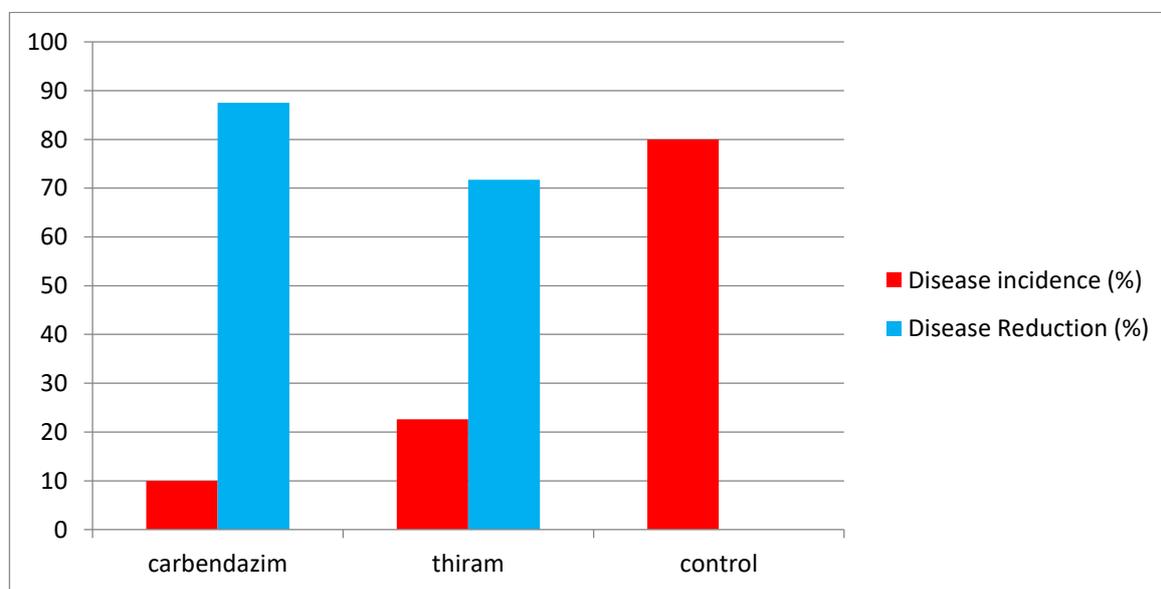
(28.70%) The per cent disease incidence in between Carbendazim and Thiram were at par to each other and maximum disease incidence was found in Thiram (28.70%) (Table 4 and Fig. 4).

The maximum disease reduction was found in Carbendazim (84.44%) followed by Thiram (68.11%) The per cent disease incidence in between Carbendazim and Thiram were at par to each other and minimum disease reduction was found in Thiram (68.11%) (Table 4 and Fig. 4).

**Table 3. Effect of Chemicals against *Cercospora canescens* on disease incidence and disease reduction *In vivo* at 30 days after sowing**

Fungal antagonist	gm/kg of seed	Disease incidence(%)	Disease reduction(%)
Carbendazim	2.0	10.00	87.50 (69.74)
Thiram	1.0	22.60	71.75 (58.01)
Control	-	80.00	0.00 (0.00)
SEm±	-	4.91	3.08
CD at 5%	-	19.28	12.10

Figure given in parenthesis are transformed value

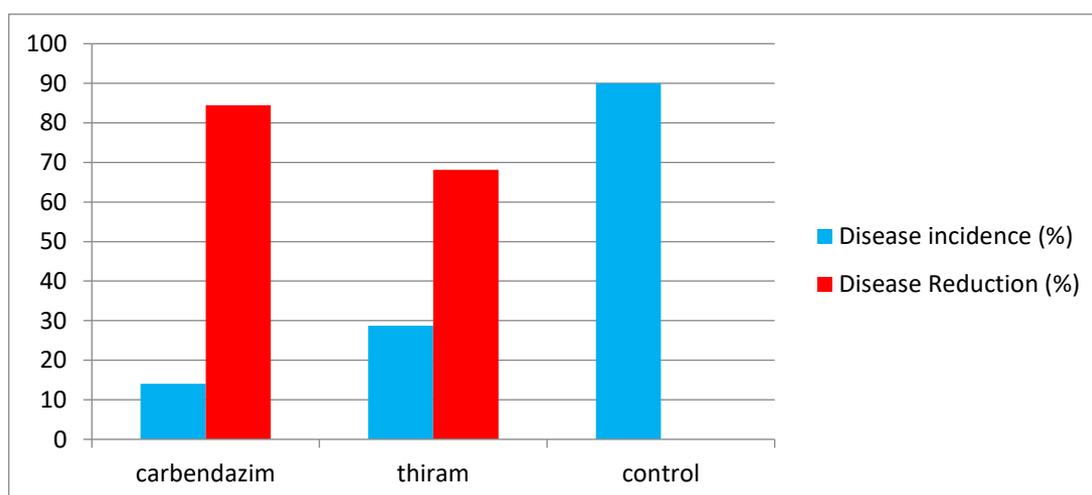


**Fig. 3. Effect of Chemicals against *Cercospora canescens* on disease incidence and disease reduction *In vivo* at 30 days after sowing**

**Table 4. Effect of Chemicals against *Cercospora canescens* on disease incidence and disease reduction *In vivo* at 45 days after sowing**

Fungal antagonist	gm/kg of seed	Disease incidence(%)	Disease reduction(%)
Carbendazim	2.0	14.00	84.44(67.00)
Thiram	1.0	28.70	68.11(55.66)
Control	-	90.00	0.00(0.00)
SEm±	-	5.53	2.24
CD at 5%	-	21.72	8.78

Figure given in parenthesis are transformed value



**Fig. 4. Effect of Chemicals against *Cercospora canescens* on disease incidence and disease reduction *In vivo* at 45 days after sowing**

Results clearly indicated that Carbendazim was better as compare to Thiram in reducing disease and enhancing plant disease reduction.

Results indicated a gradual decrease in disease incidence in all the treatments by fungicides. Most effective treatment was found Carbendazim followed by Thiram.

Dubey and Singh [5] determined “the efficacy of integrating insecticides, fungicides (carbendazim and thiram) and biological control agents (*Trichoderma viride*) as seed treatment or foliar sprays in controlling cercospora leaf spots (*Cercospora canescens* and *Pseudocercospora cruenta*) and yellow mosaic (Mungbean yellow mosaic virus) infecting urdbean (*Vigna mungo*). The combination of seed soaking in 0.1 per cent imidacloprid for 2 h and dry seed treatment with carbendazim 50 WP + thiram 75WP (1:1) 2 g/kg seed followed by foliar sprays of 0.02 per cent imidacloprid and 0.05 per cent carbendazim at 30 and 45 days after sowing, respectively, was the best treatment to control cercospora leaf Spots (4.3%) and yellow mosaic (MYMV) (9.5%), consequently resulting in the highest grain yield (954.2 kg/ha)”.

Mian et al. [6] determined “the effect of foliar application of Carbendazim (0.05%), Mancozeb (0.2%) and Urea (1.2%) on the incidence and severity of cecospora incidence and Severity of cercospora leaf spot caused by *Cercospora cruenta* and *C. canescens* and yield of mungbean. Both fungicides significantly reduced disease incidence and increase the yield however, Carbendazim was more efficient than Mancozeb”.

Khalil and Jalaluddin [7] evaluated “foliar fungicides i.e., Bavistin [carbendazim] 50 WP, Dithane M-45 [mancozeb], Indofil M-45 [mancozeb + thiophanate-methyl], Ridomil MZ [mancozeb + metalaxyl] 72, Knowin 50 WP and Thiovit [sulfur] consistently reduced the incidence and severities of cercospora leaf spot (*Cercospora cruenta*) and powdery mildew (*Erysiphe polygoni*) of black gram (*Vigna mungo*) and increased seed yield. Among these foliar fungicides, Bavistin 50 WP was the best one for the economic management of both the diseases and gave the highest seed yield followed by Knowin. The benefit - cost ratio was highest for Bavistin followed by Knowin”.

It is clear from the ongoing discussion that the two fungicides, evaluated were found effective in order to manage cercospora leaf spot diseases. But the fungicide (Vitavax) was not much more effective against cercospora leaf spot disease.

### 3.4 Efficacy of Bio-Agents Against *Cercospora canescens* on Disease Incidence and Disease Reduction *In vivo*

The results revealed that *Trichoderma viride*, *Trichoderma harzianum* significantly reduced disease incidence at 30 and 45 days after sowing.

#### ➤ At 30 days after sowing

The disease incidence was minimum in *Trichoderma viride* (16.70 %) followed by *Trichoderma harzianum* (31.40 %). The disease incidence in control was (80.00 %) at 30 days

after sowing, respectively which is differed significantly to each other. (Table 5 Fig. 5).

The maximum disease control was obtained in *Trichoderma viride* (79.13 %) followed by *Trichoderma harzianum* (60.75 %) at 30 days after sowing which is significantly, differed with each other.

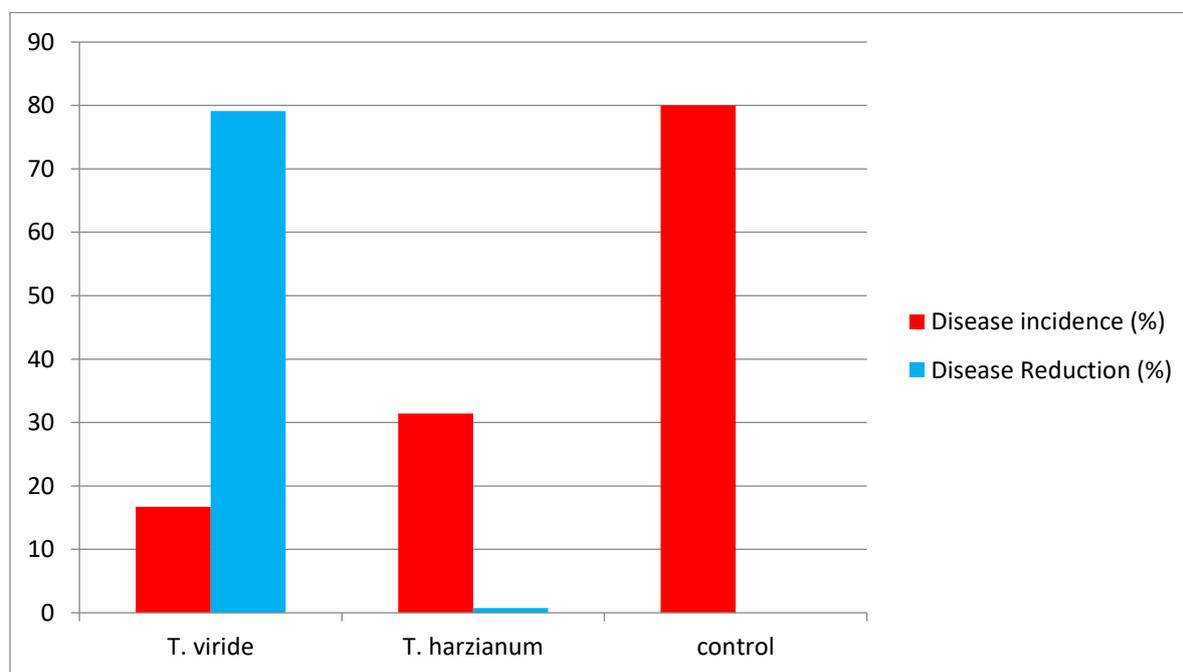
➤ **At 45 days after sowing**

The disease incidence was minimum in *Trichoderma viride* (18.30 %) followed by *Trichoderma harzianum* (35.60 %). The disease incidence in control was (90.00 %) at 45 days after sowing, respectively which is differed significantly to each other. (Table 6 and Fig. 6).

**Table 5. Effect of bio-agents against *Cercospora canescens* on disease incidence and disease reduction *In vivo* at 30 days after sowing**

Fungal antagonist	Concentration (%)	Disease incidence(%)	Disease reduction(%)
<i>Trichoderma viride</i>	10	16.70	79.13 (62.83)
<i>Trichoderma harzianum</i>	10	31.40	0.75 (51.21)
Control	-	80.00	0.00 (0.00)
SEm±	-	1.12	0.79
CD at 5%	-	4.39	3.11

figure given in parenthesis are transformed value

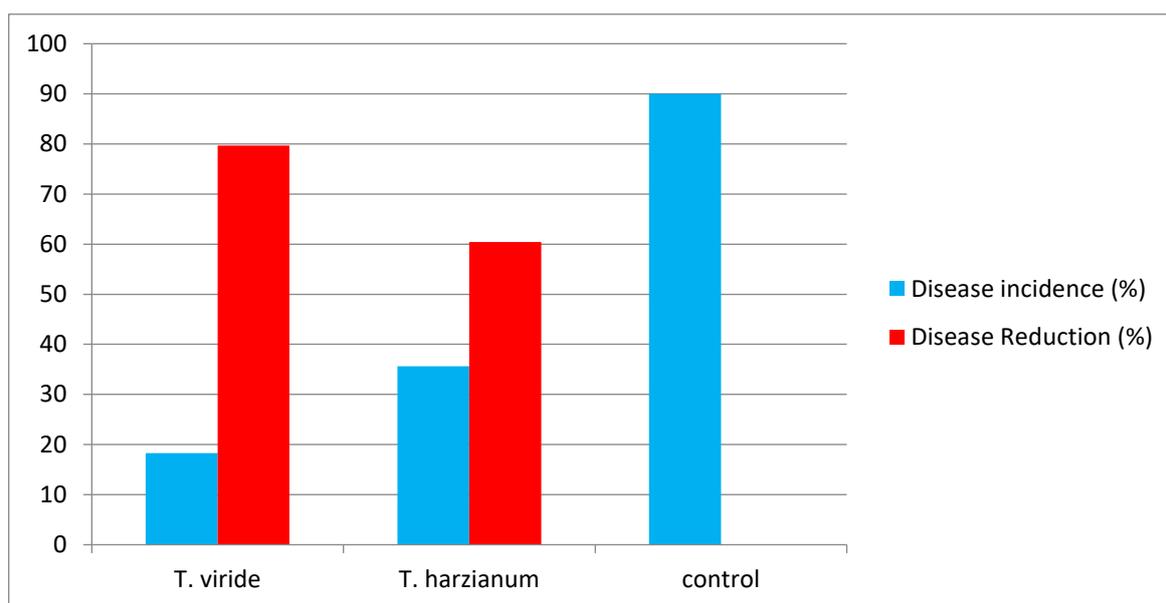


**Fig. 5. Effect of bio-agents against *Cercospora canescens* on disease incidence and disease reduction *In vivo* at 30 days after sowing**

**Table 6. Effect of bio-agents against *Cercospora canescens* on disease incidence and disease reduction *In vivo* at 45 days after sowing**

Fungal antagonist	Concentration(%)	Disease incidence(%)	Disease reduction(%)
<i>Trichoderma viride</i>	10	18.30	79.67(63.29)
<i>Trichoderma harzianum</i>	10	35.60	60.44(51.11)
Control	-	90.00	0.00(0.00)
SEm±	-	1.99	2.65
CD at 5%	-	7.80	10.39

Figure given in parenthesis are transformed value



**Fig. 6. Efficacy of bio-agents against *Cercospora canescens* on disease incidence and disease reduction *In vivo* at 45 days after sowing**

The maximum disease control was obtained in *Trichoderma viride* (79.67 %) followed by *Trichoderma harzianum* (60.44 %) at 45 days after sowing which significantly, differed with each other. (Table 6 and Fig. 6).

“Results clearly indicated that *Trichoderma viride* was better as compare to *Trichoderma harzianum* in reducing disease and enhancing plant disease reduction”. [8]

The disease control was 79.67 and 60.44 per cent in *T. viride* and *T. harzianum*, respectively which were at par to each other. Thus, the effect of bio-agents were very less when applied as foliar spray. This might be due to that the condition was not favorable for increasing the population of bio-agents on the plants. As the bio-agents is mostly soil inhabitant and grow saprophytically in presence of high organic matter in soil and increase their population very fast.

Upma Singh. [9] “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”.

In present findings the bio-agents were not much effective against *Cercospora canescens* of mungbean when applied as foliar application.

#### 4. CONCLUSION

The efficacy of botanicals Five plant extracts viz., Neem, Garlic, Tulsi, Onion, Ginger were teste *in vitro* against *Cercospora canescens*. Ten percent concentration of plant extract was found most effective *in vitro* and was further tested *in vivo* to find out the efficiency of the 5 plant extract at 30 and 45day after sowing. Two treatments viz., Carbendazim and Thiram were also tested *in vivo* against *Cercospora canescen*. All the two treatment showed significant reduction in radial growth of test fungus at different concentration.

In conclusion, mungbean stands as a vital protein source in the diets of many vegetarians across India, offering a plethora of nutritional benefits alongside its versatility. However, the prevalence of pathogens, particularly the fungal disease *Cercospora* leaf spot caused by *Cercospora canescens*, poses a significant challenge to mungbean cultivation worldwide. This disease not only leads to considerable yield losses but also affects the overall health and productivity of the crop. Integrated disease management strategies, encompassing cultural practices and the utilization of resistant or tolerant varieties, have proven effective in mitigating the impact of *Cercospora* leaf spot. Furthermore, with the current shift towards zero-budget natural farming systems and the emphasis on indigenous technologies for disease management, exploring alternative methods

becomes crucial. The present study delves into the application of botanical extracts, bio-agents, and fungicides to combat *Cercospora* leaf spot in mungbean cultivation. By assessing the efficacy of various plant extracts such as neem, onion, and garlic, alongside conventional fungicides and bio-agents, this research aims to provide valuable insights into sustainable disease management practices. By integrating multiple control techniques and prioritizing ecological principles, farmers can effectively limit the effects of *Cercospora* leaf spot, increase crop output, and ensure the long-term sustainability of mungbean production systems. This holistic approach not only safeguards crop yields but also promotes environmental stewardship and resilience in agricultural practices.

## ACKNOWLEDGEMENT

Highly acknowledgement of Dr. Subhash Chandra advisor of this research work for proper guidance and designing of this work. Department of Plant Pathology of A.N.D.U.A.T, Kumarganj, Ayodhya is highly acknowledged for providing platform and assisting from all the side to carry out this research work.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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