Comprehensive Disease Management Strategy for Cercospora Leaf Spot in Mungbean: Assessment of Botanicals, Fungicides, and Bio-Agents In Vivo

ABSTRACT

Aim: Worldwide mungbean farming is seriously threatened by the fungal disease *Cercosporacanescens*, 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.

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 *Cercosporacanescens*.
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 *Cercosporacanescens*

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.

KEY WORDS:*Cercosporacanescens*, antifungal, botanical products, mungbean.

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 (Mishra *et al*.,2022).

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 spotcaused by *Cercosporacanescens*, is a prevalent and economically significant disease affecting mungbean (*Vigna radiata*) cultivation worldwide (Sahoo *et al* 2022). 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 (Gupta and Kumar, 2020). Additionally, planting resistant or tolerant mungbean varieties offers an effective and environmentally sustainable strategy to mitigate the impact of Cercospora leaf spot (Sahoo *et al* 2022).

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.

MATERIAL AND METHODS

The present investigation were carried out during kharif 2019 at students instructional farm of A.N.D. University of Agriculture and Technology Kumarganj, Ayodhya (U.P.) India located at latitude 26.47 ^{0}N, longitude 82.12 ^{0}E and altitude 113m 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 below.

Detail of Treatment

Treatment	Biological extract	Quantity
T_1	Neem leaf extract (*Azadirachta indica*)	10 ppm/kg seed
T_2	Garlic bulb extract (*Allium sativum*)	10 ppm/kg seed
T_3	Tulsi extract (*Ocimum sanctum*)	10 ppm/kg seed
T_4	Onion extract (*Allium cepa*)	10 ppm/kg seed
T_5	Ginger extract (*Zingiber officinale*)	10 ppm/kg seed
T_6	*Trichoderma viride*	4g / kg seed
T_7	*Trichoderma harzianum*	4g / kg seed
T_8	Carbendazim	2g/ kg seed
T_9	Thiram	1g / kg seed
T_{10}	Untreated (Check)	

Application of fungicide against *Cercosporacanescens*in *In Vivo*

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

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 10treatment including control.

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.

$$\text{Percent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

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

Where,

C = Per cent disease incidence of control plots

T = Per cent disease incidence in treated plots

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 ± 2^0C 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.

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 plotsas described earlier in three replications. The sowing of untreated seeds served as check. Disease screening was recorded at 30 and 45 days after sowing.

RESULTS AND DISCUSSION

Effect of plant-derived extracts on *Cercosporacanescens* 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 (Table1 and fig. 1).

Table 1. Effect of plant extracts on disease incidence and disease reduction against *Cercosporacanescens* 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

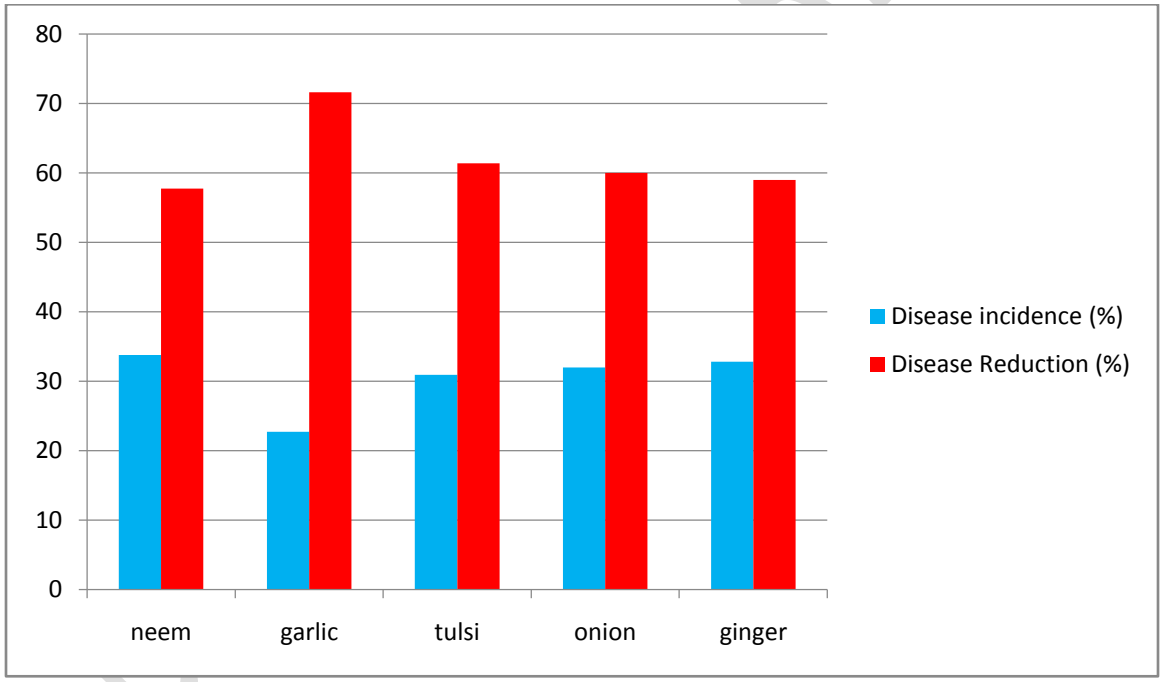

Fig. 1: Effect of plant extracts on disease incidence and disease reduction against *Cercosporacanescens* mungbean in vivo at 30 days after sowing.

- **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.

Table 2. Effect of plant extracts on disease incidence and disease reduction against *Cercosporacanescens*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

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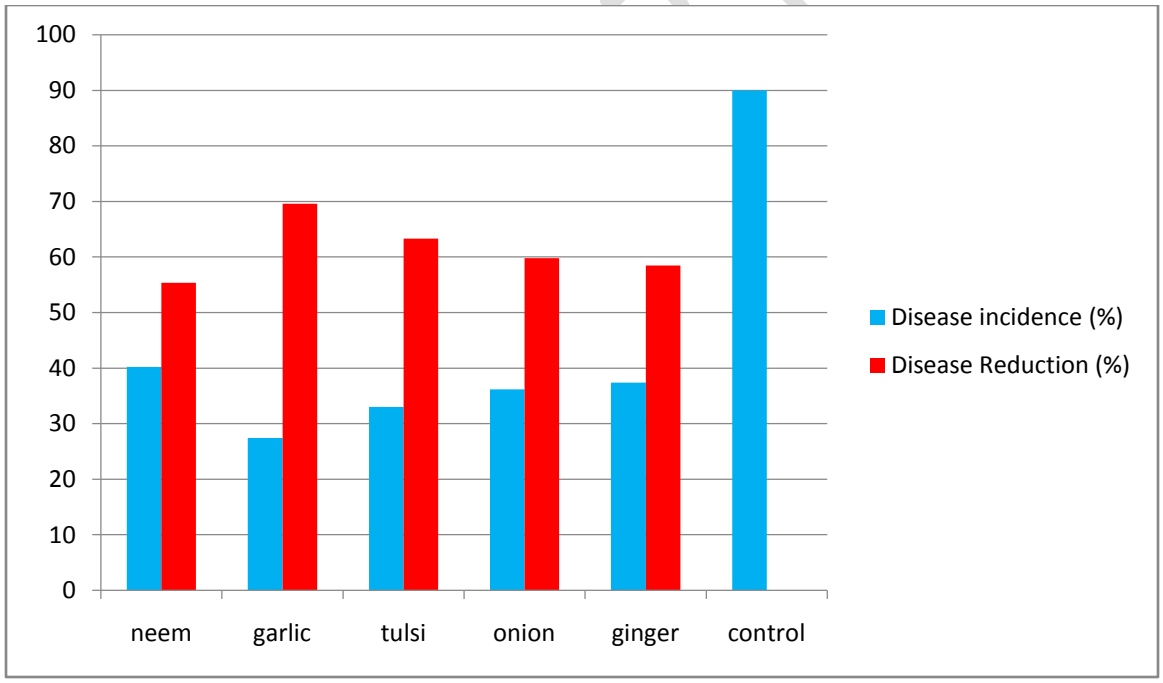

Fig. 2: Effect of plant extracts on disease incidence and disease reduction against *Cercosporacanescens*mungbean*in vivo* at 45 days after Sowing.

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. Per cent disease reduction in rest of the treatment differed significantly (Table 2 and fig. 2).

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 (leafextracts) applied as foliar spray in controlling cereospora leaf spot of groundnut in two successive growing seasons. All the plant extracts reduced the incidence and severity of cercospora leaf spot in both seasonscompared to the untreated crops. However, Neem (seed) and Garlic (clove) extracts significantly reduced the incidence and severity of thedisease 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 *Cercosporacanescens*. *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.

Efficacy of chemicals against *Cercosporacanescens* 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).

Table 3.Effect of Chemicals against *Cercosporacanescens* 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

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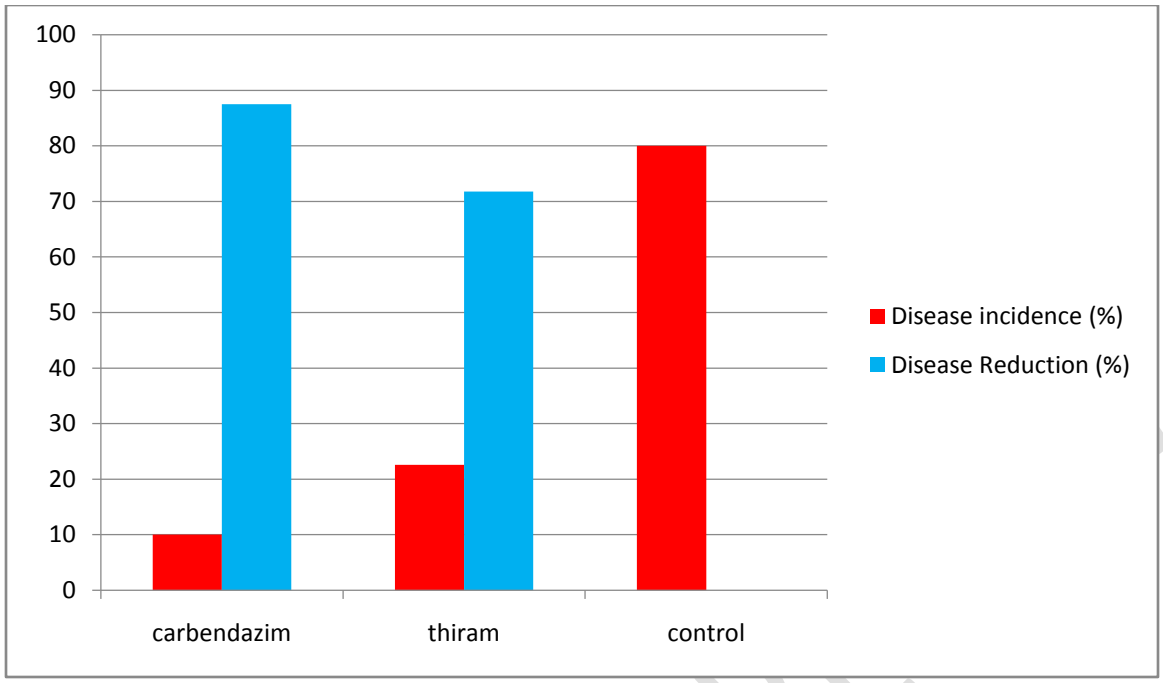

Fig. 3: Effect of Chemicals against *Cercosporacanescens* on disease incidence and disease reduction *in vivo* at 30 days after sowing

- **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 4. Effect of Chemicals against *Cercosporacanescens* on disease incidenceand 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

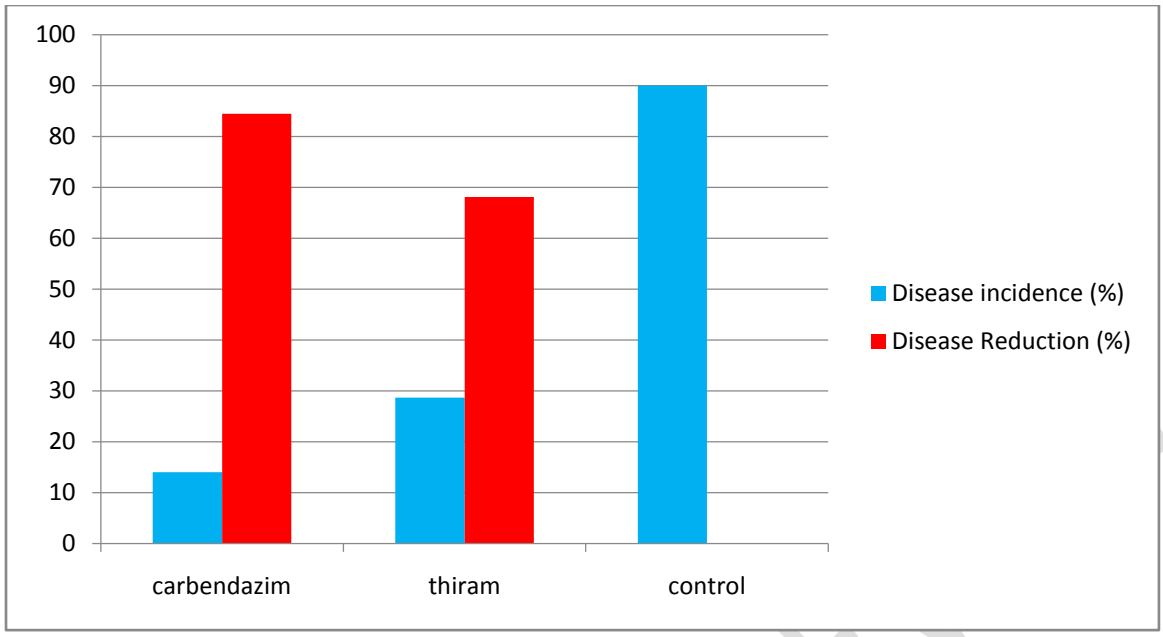

Fig. 4: Effect of Chemicals against *Cercosporacanescens* on disease incidence and disease reduction *in vivo* at 45 days after sowing

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 (2006) determinated 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 *Pseudocercosporacruenta*) 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 resultingin the highest grain yield (954.2 kg/ha).

Mian *et al.* (2000) determined the effect of foliar application of Carbendazim (0.05%), Mancozeb (0.2%) and Urea (1.2%) on the incidence and severity of cecosporaincidence and Severity of cercospora leaf spot caused by *Cercosporacruenta* and *C.canescens* and yield of mungbean. Both fungicidessignificantly reduced disease incidence and increase the yield however,Carbendazim was more efficient than Mancozeb.

Khalil and Jalaluddin (2004) evatuated 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 incidenceand severities of cercospora leaf spot (*Cercosporacruenta*) and powderymildew (*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 thehighest 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 spotdiseases. But the fungicide (Vitavax) was not much more effective against cercospora leaf spotdisease.

Efficacy of bio-agents against *Cercosporacanescens* 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).

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

Fungal antagonist	Concentration (%)	Disease incidence (%)	Disease reduction (%)
Trichoderma viride	10	16.70	79.13(62.83)
Trichoderma harzianum	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

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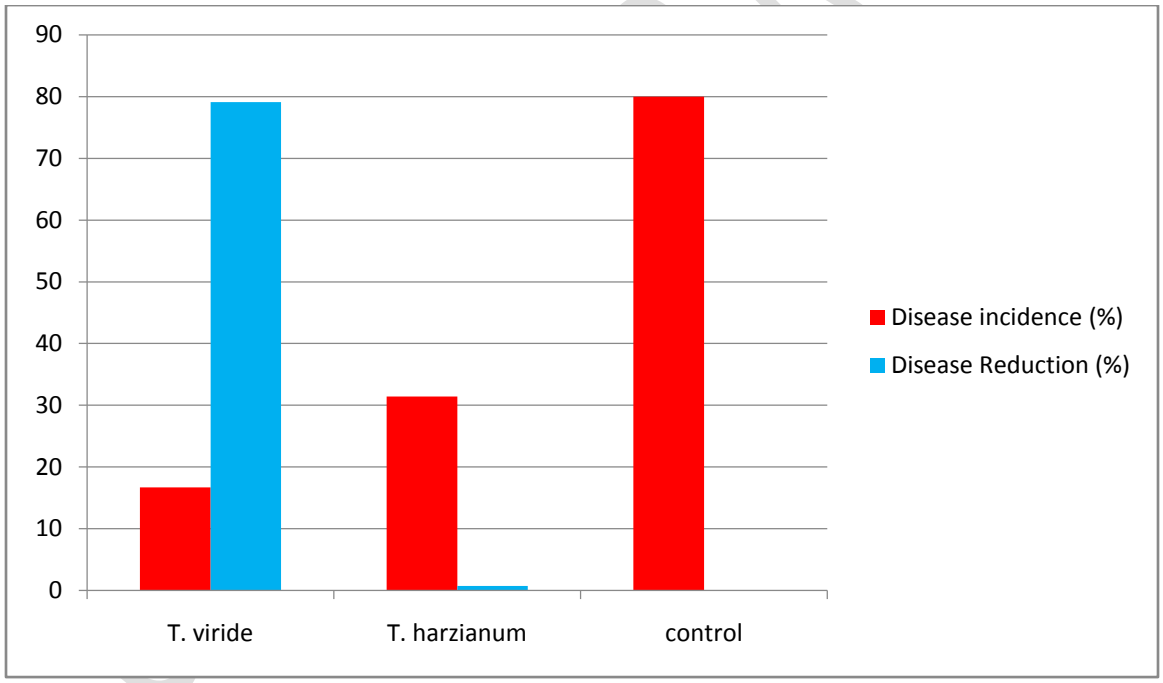

Fig. 5: Effect of bio-agents against *Cercosporacanescens* on disease incidence and disease reduction *in vivo* at 30 days after sowing

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 6.Effect of bio-agents against *Cercosporacanescens* on disease incidence and disease reduction *in vivo* at 45 days after sowing

Fungal antagonist	Concentration (%)	Disease incidence (%)	Disease reduction (%)
Trichoderma viride	10	18.30	79.67 (63.29)
Trichoderma harzianum	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

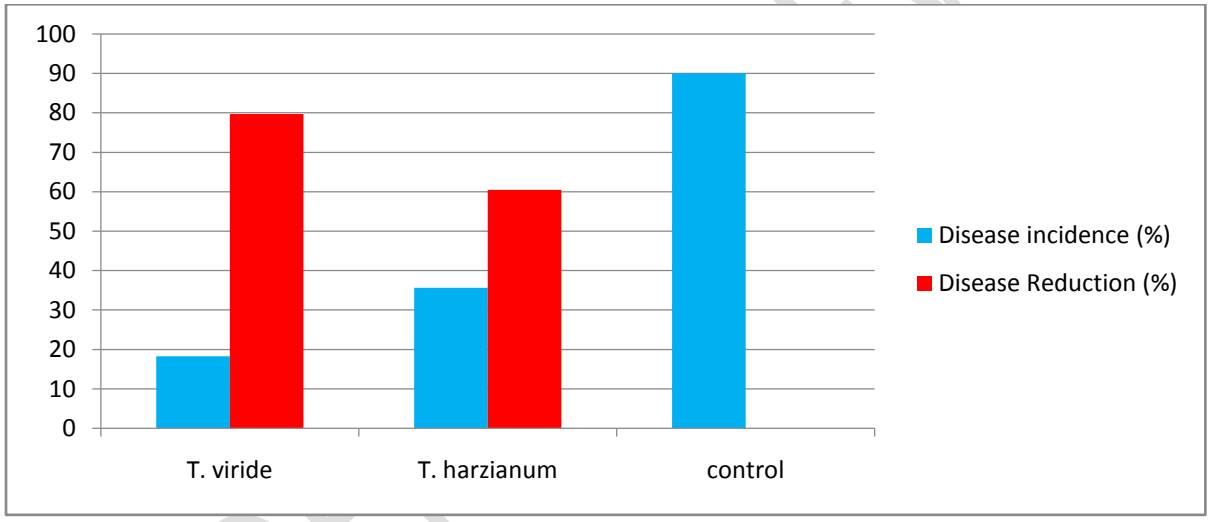

Fig. 6: Efficacy of bio-agents against *Cercosporacanescens* on disease incidence and disease reduction *in vivo* at 45 days after sowing

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.

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., (2015) an extensive survey of Kota district resulted several fungal diseases occurring on Trigonella crop. Leaf spot caused by *Cercosporatraversiana* 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 *Cercosporacanescens* of mungbean when applied as foliar application.

CONCLUSION

The efficacy of botanicals Five plant extracts *viz.,* Neem, Garlic, Tulsi, Onion, Ginger were teste*in vitro* against *Cercosporacanescens.*Tenpercent 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 *Cercosporacanescen.* 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 Cercosporacanescens, 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.

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Authors contribution

The work was performed by RSR. NS has assist in manuscript drafting. VS and GP has helped in manuscript preparation.

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