Research Article

# Evaluation of Advanced Mung Bean Germplasm against *Cercospora* Leaf Spot and its *In-vitro* Management by Different Fungicides

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**Abstract** | Mung bean (*Vigna radiata* (L.) R. Wilczek var. *radiata*)/ green gram is one of the short period legume and poor man protein source along with carbohydrates and vitamins. Cercospora Leaf Spot is a devastating threat to the crop caused by *Cercospora canescens,* affects the whole crop and 95% yield losses may be attributed in severe conditions. To manage the diseases through tolerant germplasm and with environmentally safe fungicides is a cost-effective approach. The present study was aimed to find the resistant germplasm against the disease and to evaluate the relative efficacy of different fungicides against the *C. canesens* in lab conditions. Twenty varieties/lines were screened against Cercospora Leaf Spot disease in field conditions. No line/ variety was found immune and only one line (16051) was resistant against this disease. Five different fungicides Cymoxanil 8% + Mancozeb 64% (Curzate), Copper Hydroxide (Champion), Thiophanate-Methyl (Topsin-M), Pyraclostrobin + Metiram (Cabriotop), and Mifenoconazole (Score) were evaluated at 10, 50, 100, and 200 ppm concentration against *C. canescens* using poisoned food technique. Score at 100 ppm was found most effective (93.03%) against the disease as compared with other fungicides. From present research, it is devised that 16051 line may be used for future breeding program and Score at 100 ppm may be evaluated for field trials in future studies.

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## Introduction

Mung bean (*Vigna radiata* L.) also known as "green-gram" is a short-term legume of family Fabaceae that enriched with proteins, carbohydrates and vitamins (Hassan *et al*., 2017). Mung bean as a dry seed is one of the major pulse crops of south and southeast Asia and the excellent source of plant protein. The dried seeds of green gram contain 23.6% protein, 58.2% carbohydrates, 1.2% fats, 3.3% fibers, 5.9-7.6 mg/100 g iron and 4% minerals (Dahiya *et al*., 2015; Chavhan *et al*., 2018). Recently, mung bean was grown on 161.8 thousand ha and production was 118.8 thousand tons in Pakistan which is less than the previous year (2017-18). Globally, the annual mung bean yield was about 5.3 million tonnes, harvested at 7.3 million ha area (2015-2017) (Anonymous, 2019).

Mung bean is vulnerable to many viral, bacterial and fungal disease under conducive environment (Mian, 1976; Rai and Mamatha, 2005). *C. canescens* causes colossal losses to yield in major Mung bean growing areas. *C. canescens* affects the foliation and eventually damaged whole crop quality that lead to yield loss up to 50-70% (Lal *et al*., 2001; Chand *et al*., 2012). At optimum temperature (25-30 °C) and relative humidity (90-100 %) the disease losses due to this fungus were observed 90-100 % in Pakistan (Shahbaz *et al*., 2014).

Initially, the brownish spots appear on leaves lately on branches, stem and pods too that ultimately reduce the number/ size of pods and seeds (Munjal *et al*., 1960; Grewal *et al*., 1980). There are many strategies for this disease management like tillage operations, crop rotation, host plant resistant, infrared radiations and fungicides (Hanson and Panella, 2003; Khan and Smith, 2005; Khan, 2008; Meenu *et al*., 2018). Fungicides (Captain and Carbendazim, Thiamethoxan + Carbendazim) were assessed and found good for disease management and increased yield (Bhat *et al*., 2015). The valued act of disease management is resistant/ tolerant breeding material. In mung bean, there was breeding material/ cultivars evolved through different breeding/ recombinant lines, land races and wild relatives. Such derived breeding material needs to be evaluated against different diseases to configure the resistant or susceptible lines/ cultivars. On the other hand, use of environmentally safe chemicals are the common and traditional practice to tackle with fungal diseases. So, the present study was conducted to evaluate the response of mung bean germplasm and different fungicides for the management of leaf spot disease caused by *C. canesens*.

## Materials and Methods

### *Evaluation of mung bean germplasm against C. canescens*

The high humid and dry Kharif season was selected to conduct the experiments in the fertile soil of Pulses Research Institute Faisalabad, Pakistan. The 20 germplasm entries (16004, 16005, 16015, 16016, 16017, 16019, 16020, 16022, 16023, 16024, 16039, 16051, 16053, 16058, 16059, 16064, 16071, 16078, 16090, 16091) were evaluated at Pulses Research Institute, AARI, Faisalabad. Seeds were sown with dibblers at 30 cm row to row and 10 cm plant to plant distance. All agronomic practices were done. The trial was conducted in augmented design and a susceptible check $C_2$-94-4-36 was sown after two entries.

The inoculum was prepared on Potato Dextrose Agar (PDA) medium by refreshing the old culture already available at Plant Pathology Lab, Pulses Research Institute, AARI Faisalabad, Pakistan. The mass multiplication of *C. canescens* for germplasm evaluation was done on mung bean seeds as described by Shahbaz *et al*. (2014). The inoculum was sprayed on plants at $5\times10^5$ conidia/ml concentration (Shahbaz *et al*., 2014). The plants were observed for symptoms development regularly. Disease severity of *C. canescens* on mung bean germplasm was recorded after appearing the symptoms according to the 0-9 rating scale (Mayee and Datar, 1986) (Table 1).

### *In vitro efficacy of different fungicides against C. canescens*

The poisoned food technique was used to evaluate the different fungicides cymoxanil 8% + mancozeb 64% (Curzate), copper hydroxide (Champion), thiophanate-methyl (Topsin-M), pyraclostrobin + metiram (Cabriotop), and difenoconazole (Score) at 10, 50, 100, and 200 ppm concentration against *C. canescens*. In the control treatment, no fungicide at any concentration was applied. The plates were wrapped and incubated at 25 ± 2 °C. The effectiveness of fungicides was measured by growth inhibition zones of *C. canescens* over control by the following formula (Vincent, 1927).

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

Where;
I= Percentage inhibition; C= growth in control and T= growth in each treatment.

### *Data analysis*

The recorded data were analyzed using ANOVA and differences among the means were partitioned at $P$=0.05 according to Least Significant Difference (LSD) test by using Statistix (v 8.1) software.

## Results and Discussion

### *Germplasm reaction against Cercospora leaf spot disease*

According to the disease rating results, no line was found immune or highly resistant against the disease. Only one line (16051) was found resistant, and 16004, 16005, 16015, 16016, 16017, 16019, 16020, 16023, 16024, 16039, 16053, 16058, 16059,


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16064, 16071, 16090, 16091 were found moderately resistant. Two lines (16022, 16078) were found susceptible. No line exhibited a highly susceptible response (Table 2).

*Efficacy of different fungicides against C. canescens under lab conditions*

Data revealed that Score was the most effective against this pathogen with 14 (10 ppm dose), 10 (50 ppm dose) and 0 % (100 ppm dose) mycelial growth in comparison to the control treatment. The best concentration of Score was 100 ppm where it limited 100% pathogen's growth. Whereas, the overall disease inhibition percentage by Score was 93.03%. While, Cabriotop was also good at 100 and 200 ppm by restricting the growth at 6.60 and 3.20% levels, respectively when compared with control growth. Overall, Cabriotop concentrations showed 86.63 % inhibition zone which was less than Score. On the other hand, Topsin-M, Champion and Curzate showed 14.60, 33.60 and 43.80 % mycelial growth at 200ppm, respectively (Table 3).

Mung bean is an important legume crop worldwide and used due to its nutritional importance. There are various diseases of mung bean that play important role in reducing the yield. But the cercosporin toxin, a photo-activator induce *Cercospora* leaf spot disease in mung bean. A number of ways are being used by researchers to manage the disease in the field. Resistant verities and use of chemicals are of major concern in this respect. The disease management practices and resistant germplasm are vital for tackling the yield loss problems in mung bean. This research was conducted to study the response of available germplasms to the *Cercospora* leaf spot disease and to evaluate the response of fungicides against pathogen *in vitro* conditions. Also, the study of multi-environment involve with genotypes are the best way to adopt a germplasm in an area. Through, GGE biplot two genotypes LGG 460 and COGG 912 were found best out of 246 and bearing durable resistance against *Cercospora* leaf spot disease (Das *et al*., 2019). Such durable resistant material is the excellent source of future breeding and research programs to manage *Cercospora* leaf spot disease of mung bean.

Here in this research, the mung bean germplasm material 16004, 16005, 16015, 16016,16017, 16019, 16020, 16023, 16024, 16039, 16053, 16058, 16059, 16064, 16071, 16090, 16091 were found moderately resistant with unique resistant line 16051. These are the Pakistani mung bean breeding material that can be used in future breeding programs to cope with disease problems in the world. In the meanwhile, India hold about 100 resistant gene pole material related to CLS disease that can be a major source of breeding research in future. Such resistant germplasm can be collected and adopted in the region for betterment of mankind and to develop further resistant varieties (Kumar *et al*., 2020). Two populations BC1F and F2 were evaluated under many field and environmental conditions. And 69 markers were used to study the polymorphism that revealed the single gene dominant resistance in two populations. Furthermore, composite gap mapping analysis showed one big quantitative trait loci (QTL) for this disease in two populations (Chankaew *et al*., 2011). By interval gene mapping using different markers (ISSR, ISSR-RGA and SSR), QTL were studied and found 79.8 % variability that revealed the resistance position of CLS (qCLSC72V18-1) between VR393 and I16274 marker. So, the use of closely related markers of CLS resistant gene will be a best choice to map more resistant genes in mung bean (Tantasawat *et al*., 2020).

The defences mechanism *in vivo* and *in vitro* was also found interesting by analysing the PR Proteins, PAL and phytoalexin genestein in mung bean. These were found as biochemical markers against *Cercospora* disease and also helpful in initial evaluation of germplasm (Koche and Chaudhary, 2019). There were number of superoxide dismutase and catalase enzymes that were found actively involved in mung bean resistance against *C. canescens*. These enzymes involve in detoxification of cercosporin too that might help in resistant mechanism of the plant against pathogen attack. On the basis of this mechanism many recombinant inbred lines (RILs) were identified that showed resistant against *Cercospora* leaf spot disease (Maurya *et al*., 2018). In another study, among 16 germplasm accessions, VC-6153-B-20 was found resistant at rainfed regions of Bangladesh. Meanwhile, secure 660WG was showed good results in *Cercospora* disease management and increased the yield as compared with control treatment (Hasan *et al*., 2017). The genetic approaches with conventional breeding program will be the better option to tackle the problem soon.

**Table 1:** *Disease severity scale for rating of Cercospora leaf spot.*

| Ratings | Symptoms on plants | Reaction |
|---|---|---|
| 0 | No visible symptoms on plants | Immune (I) |
| 1 | 1-10% foliage or pod area affected with small pinhead lesions | Highly Resistant (HR) |
| 3 | 11-20% foliage or pod area affected with small round brown spots | Resistant (R) |
| 5 | 21-30% foliage or pod area affected with large spots | Moderately Resistant (MR) |
| 7 | 31-50% foliage or pod area affected with bigger coalescing spots | Susceptible (S) |
| 9 | 51-100% foliage or pod area affected with bigger coalescing spots | Highly susceptible (HS) |

**Table 2:** *Response of mung bean germplasm against Cercospora leaf spot.*

| Rating | Genotype response* | Genotype | No. of genotype |
|---|---|---|---|
| 0 | Immune | 0 | 0 |
| 1 | Highly resistant | 0 | 0 |
| 3 | Resistant | 16051 | 1 |
| 5 | Moderately resistant | 16004, 16005, 16015, 16016,16017, 16019, 16020, 16023, 16024, 16039, 16053, 16058, 16059, 16064, 16071, 16090, 16091 | 17 |
| 7 | Susceptible | 16022, 16078 | 2 |
| 9 | Highly susceptible | 0 | 0 |
| Total | | | 20 |

**Reaction based on percent disease severity.*

**Table 3:** *Fungicides effectiveness against C. canescens.*

| Fungicide | Mycelial growth (mm) | | | | Mean MG (mm) | GI % |
|---|---|---|---|---|---|---|
| | 10 ppm | 50 ppm | 100 ppm | 200 ppm | | |
| Curzate | 82.20±2.13b[1] | 67.40±0.60b | 55.20±0.49b | 43.80±0.37b | 62.15 | 30.76 |
| Champion | 74.40±0.60c | 61.00±0.63c | 46.80±0.80c | 33.60±0.40c | 53.95 | 39.90 |
| Topsin-M | 64.20±0.58d | 43.60±0.40d | 28.20±0.49d | 14.60±0.75d | 37.65 | 58.05 |
| Cabriotop | 23.80±0.37e | 14.40±0.75e | 6.60±0.40e | 3.20±0.37e | 12.00 | 86.63 |
| Score | 14.60±0.75f | 10.40±0.40f | 0.00±0.00f | 0.00±0.00f | 6.25 | 93.03 |
| Control | 89.60 a | 90.00 a | 89.80 a | 89.70 a | 89.77 | |
| CV | 4.70 | 3.25 | 4.14 | 5.25 | | |

*[1]Means within a column sharing the same letter are not significantly different from each other at P = 0.05 according to Least Significant Difference Test; MG: Mycelial growth; GI: Growth inhibition.*

Despite of breeding strategies, the chemical management of *Cercospora* leaf spot is an important factor to confront this challenge. Score and Cabriotop fungicides were found and recommended best for the management of this disease in dry wet conditions. When the early symptoms appear, one spray of Ridomil gold was also a good choice with dose 618g/ha in many areas of Pakistan (Shahbaz *et al*., 2014). Awasthi *et al*. (2018) studied the fungicides effect on mung bean crop to control the foliar disease leaf spot. They conclude that the mixture of Indofil M45 with Hexaconazol plus Mancozeb decreased the disease incidence to best extent (Awasthi *et al*., 2018). Hexaconazol, alone was also found effective results against *Cercospora* leaf spot (Hegde *et al*., 2013). The yield of legumes increased significantly by applying Mancozeb to manage the foliar disease caused by *Cercospora* spp (Sheriff and Turaki, 2019). Singh *et al*. (2013) approved that Propiconazole and Carbendazim were not only completely stopped the mycelial growth of *Cercospora in vitro*, but showed best results at greenhouse trials too (Singh *et al*., 2013). Also, bio-fungicides like plant extracts of Neem, Datura and Garlic were evaluated for *Cercospora* management. The data revealed that Garlic was performed well against the disease followed by Datura and Neem (Ramesh *et al*., 2014).


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## Conclusions and Recommendations

The study was conducted to evaluate the mung bean germplasm and to check the market available fungicides against *C. canescens*. One resistant and 17 moderately resistant lines were sorted out to release in the field for farmers and for the use of further breeding experiments. The resistant material found here, must be used in further breeding research programmes to develop durable disease resistant varieties of mung bean. Score and Cabriotop fungicides were recommended to farmers for the management of *C. canescens* that ultimate increased the yield to a good extent.

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## Novelty Statement

In this research work, we studied the effect of 5 different fungicides to manage the *Cercospora* leaf spot disease and Score found best in this regard. Also, the mung bean line 16051 was found resistant against said disease.

## Author's Contribution

**Huma Abbas and Muhammad Azhar Iqbal:** Design and supervise the research experiments.
**Muhammad Kamran and Muhammad Umar Shahbaz:** Write up the manuscripts.
**Haseeb Ullah Kamber and Muhammad Ehetisham ul Haq:** Technically evaluated the data.
**Hira Abbas and Muhammad Junaid:** Helped in data recording.
**Nazir Javed:** Helped in writeup and review the manuscript.

*Conflict of interest*
The authors have declared no conflict of interest.

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