Research Article

Unveiling the Factors Affecting Leaf Spot Disease in Mungbean and its Management

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Abstract | Mungbean (*Vigna radiata* (L.) is cultivated on large area and has high nutritional values. Leaf spot incited by *Cercospora canescens* is the major constraint that significantly affects the productivity of mungbean. This research was aimed to manage the Cercospora leaf spot diseases by using different chemicals by foliar spray using 0.05g of boric acid, $ZnSO_4$, $MgSO_4$, and a combination of all these. The *in-vivo* experiment was carried out in the open field using randomized complete block design (RCBD). Effects of different chemicals on disease management were examined statistically by using Tukey's honestly significant difference (HSD) test at 5% level of significance. The most effective treatment was the combination of all 3 chemicals that gave maximum disease control and 11.72% disease severity was recorded. Mungbean cultivars were also screened for the source of resistance against leaf spot disease and none was found immune and highly resistant. Correlation and regression analysis were performed to find the relation of environmental factors and disease development. There was a positive relationship between disease severity (*Cercospora* leaf spot disease) and relative humidity, wind speed and rainfall. The results revealed that there was negative correlation between maximum temperature and disease development. It could be concluded that the application of nutrients alone and in combination caused significant reduction in disease severity and enhanced the plant growth.

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Introduction

Mungbean (*Vigna radiata* L.), often known as green gram is a short-duration legume that belongs to the *Fabaceae* family and it has diploid chromosome (Hassan *et al*., 2017). Mungbean is significant for its high nutritional content as it has protein 20-30%, carbohydrates 58.2%, starch above 45%, minerals 4%, iron 6.8%, fats 1.2% and generally low quantity of fiber 6.5% (Dahiya *et al*., 2015; Chavhan *et al*., 2018). It converts nitrogen into its usable form through symbiosis along with rhizobium, which help the legume to meet not only its own nitrogen requirements, but also improve crops (Ali and Gupta, 2012). It also enhances soil fertility, texture and it takes less water than other legumes to grow well (Parida and Das, 2005). Mungbean can be cultivated in a variety of Pakistani environmental circumstances as it has diverse adaptability to epidemiological situations (Ghulam, 2012). Despite the crop's fast spread, production has been declining in recent years in comparison to other countries due to different biotic and abiotic factors (Partap *et al*., 2019). Mungbean development can be influenced by more than 30 phytopathogenic microorganisms, the majority of which are fungi, which can cause a remarkable reduction in quality and crop yield that depends on environmental conditions (Bhaskar, 2017). *Cercospora* leaf spot (CLS) is a significant foliar disease of mungbean caused by *Cercospora canescens*. *Cercosporoid* fungi is present in countless cultivated plants in nearly every continent (Braun *et al*., 2016). The disease was appeared first time in Delhi, India that is present worldwide where high temperature and humidity prevails (Pandey *et al*., 2018). Under natural epiphytotic environments, the pathogen causes up to 96% qualitative and quantitative damage (Shahbaz *et al*., 2014). *Cercospora canescens* affects the crop by causing round or randomly shaped leaf spots with greyish-white interiors and reddish-brown to dark brown edges (Rai and Mamatha, 2005). As the disease progresses, the tissues of infected leaves will die the foliation eventually harmed total crop quality, resulting in production losses of up to 70% (Chand *et al*., 2012). The effects of environmental aspects on plant like high and low temperature, relative humidity, wind velocity and rain definitely have a positive or negative impact on disease and both pathogens and plants (Vela´squez *et al*., 2018). Nutrients may boost the plant defense mechanism resulting in repair of damages caused by *Cercospora canescens*.

The present study was conducted with the following aims:

- To assess the resistance potential of mungbean germplasm against *Cercospora* leaf spot disease
- To evaluate different nutrients against *Cercospora* leaf spot
- To evaluate the effect of nutrients application on plant growth parameters
- To assess different weather variables favorable for the *Cercospora* leaf spot

Materials and Methods

Trial location

The trial was carried out in field area of Department of Plant Pathology, University of Agriculture Faisalabad (UAF) Pakistan. The field area was prepared by keeping in view the recommended mungbean agronomic practices maintaining 10 cm plant to plant and 30 cm row to row distance. The experiment was conducted under randomized complete block design (RCBD) with 3 replications.

Seed collection

Seeds of 11 mungbean varieties (NM-54, NM-51, NM-28, NM 121-25, NM-98, NM 20-21, NM 19-19, NM 31-1, NM-201, Kabuli mung, NM-2016) were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan).

Diseased sample collection

Mungbean leaves with typical disease symptoms (*Cercospora* leaf spot) were collected. The samples were properly labeled with the disease name, collection date, variety name and sample number after being packed in polythene bags. The specimens were taken to the plant Mycology lab of Department of Plant Pathology, UAF. The samples were kept at 4°C in refrigerator for further use.

Isolation and purification of the fungus

The fungus was isolated on PDA plates, from symptomatic mungbean leaves. The diseased leaves were washed under tap water then surface sterilized with 70% ethanol and 3 times with distilled water. The samples were blotted dry and put aseptically in autoclaved PDA medium plates. The petri plates were incubated at 25°C. The mycelium growth was picked and placed into new PDA plates for purification purposes.

Morphological identification of fungal pathogen

The colony appearance, including texture, color and the form of the hyphae and spores, was studied using a stereoscope (SWIFT Instruments, USA) for morphological identification. Mycelium was picked with a sterilized needle from the isolated plates and placed on the slide with one drop of water, covered with cover slip, and examined under microscope.

Sporulation of pure cultures was done by transferring a mycelial plug to 2% water agar (20g/L dH_2O_2) mixed with host material (mungbean leaves) to investigate colony shape (Inderbitzin et al., 2010) and plates were placed under UV light on a continual basis (Pavlic et al., 2004).

Management of disease

In order to manage the *Cercospora* leaf spot disease 4 treatments i.e., $MgSO_4$, $ZnSO_4$, H_3BO_3, $MgSO_4$+ $ZnSO_4$ + H_3BO_3, were used. The trial for disease management comprised of 4 varieties i.e., Kabuli mung, MN-28, NM-54, and NM 121-25. The treatments were sprayed at 15 days interval.

Data collection

The data of weather variables (temperature, relative humidity, wind velocity and rainfall were obtained from weather observatory, UAF. The data of disease incidence, severity, plant height, root length, fresh weight, and dry weight were recorded at 15 days interval by using below mentioned formulae.

$$\text{Disease incidence \%} = \frac{\text{No. of infected plants}}{\text{Total no. of Plants}} \times 100$$

$$\text{Disease Severity \%} = \frac{\text{No. of infected leaves}}{\text{Total no. of leaves}} \times 100$$

The data of disease severity was recorded 1 day before and 3 days after the foliar application of nutrients.

Statistical analysis

Correlation and regression analysis were performed in order to check the association of environmental variables for disease development. The data was analyzed statistically by using Fisher's analysis of variance technique and 5% probability level was used to compare the treatment means with the help of Statistic 8.1.

Results and Discussion

Evaluation of mungbean germplasm against Cercospora leaf spot disease

The results of screening showed that NM-54 and NM-51 are the resistant varieties with minimum disease severity 13.83% and 19.22 %, respectively and fall in the category of 10.1-20.0 with grade 2 of rating scale. NM-201, NM-13-1 and NM-121-25 are moderately resistant against disease with 25.37%, 26.25% and 28.16% disease severity that indicates 3rd grade and 20.1-30.0 category of scale (Table 1). The susceptible varieties according to scale are Kabuli mung, NM-20-21 and MN-28. These varieties are in 4th grade that shows 30.1-50.0 categories with 32.14%, 35.32% and 38.71% disease severity, respectively. In grade 5 with category >50.0 are NM-19-19, NM-2016 and NM-98 varieties that are highly susceptible towards the disease and shows maximum disease severity 53.84%, 56.2% and 61.96%.

Table 1: *Genotypes of mungbean evaluated for source of resistance against Cercospora leaf spot.*

Grade	Categories	Varieties	Disease severity (%)	Disease reaction
0	0	–	–	Immune
1	0.1-10.0	–	–	Highly resistant
2	10.1-20.0	NM-54 NM-51	13.83 19.226667	Resistant
3	20.1-30.0	NM-201 NM-13- 1 NM-121- 25	25.373333 26.256667 29.166667	Moderately resistant
4	30.1-50.0	Kabuli mung NM-20- 21 NM-28	32.146667 35.32 38.71	Susceptible
5	>50.0	NM-19- 19 NM-2016 NM-98	53.84 56.2 61.96	Highly susceptible

According to the above presented data NM-54 and MN-51 showed resistant response while NM-201, NM-13-1 and NM-121-25 are moderately resistant (Figure 1). Kabuli Mung, NM-20-21 and NM-28 are susceptible whereas NM-19-19, NM-2016 and NM-98 are highly susceptible varieties against *Cercospora* leaf spot disease of mungbean.

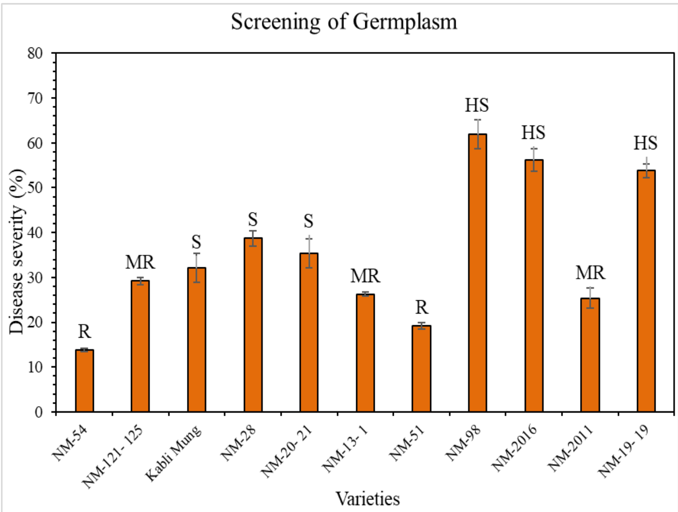

Figure 1: *Genotypes of mung bean evaluated for source of resistance against Cercospora leaf spot.*

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Table 2: *Correlations (Pearson) analysis for Cercospora leaf spot.*

	NM-28	Kabli Mung	NM-121-25	NM-54	Max. temperature	Min. temperature	Relative humidity	Rain fall
Kabli Mung P-value	0.9881 0.0000**							
NM-121-25	0.9886 0.0000**	0.9844 0.0000**						
NM-54	0.9809 0.0000**	0.9867 0.0000**	0.9823 0.0000**					
Max. temperature	-0.9938 0.0000**	-0.9865 0.0000**	-0.9959 0.0000**	-0.9829 0.0000**				
Min. temperature	-0.9915 0.0000**	-0.9821 0.0000**	-0.9966 0.0000**	-0.9785 0.0000**	0.9994 0.0000**			
Relative humidity	0.8944 0.0001**	0.9181 0.0000**	0.8524 0.0004**	0.9170 0.0000**	-0.8744 0.0002**	-0.8578 0.0004**		
Rain fall	0.9265 0.0000**	0.9455 0.0000**	0.8911 0.0001**	0.9442 0.0000**	-0.9105 0.0000**	-0.8963 0.0001**	0.9968 0.0000**	
Wind speed	0.5884 0.0441*	0.6354 0.0264*	0.5089 0.0911^{NS}	0.6249 0.0298*	-0.5439 0.0676^{NS}	-0.5157 0.0861^{NS}	0.8808 0.0002**	0.8404 0.0006**

*** Highly Significant p-value < 0.01; * Significant p-Value < 0.05; ^{NS}Non-Significant p-Value > 0.05*

Effect of environmental variables on the Cercospora leaf spot disease development

The results of overall correlation of environmental conditions on *Cercospora* leaf spot for 4 different varieties of mung bean (Kabuli mung, NM-121-25, NM-28, NM-54) showed that each variety had a different relationship of disease severity with the metrological factors. The relationship between disease severity and temperature (maximum and minimum) is negative indicating that there was significant decrease in disease severity with increase in maximum and temperature. The mean values 0.8944, 0.9181, 0.8524, 0.9170 for NM-28, Kabuli mung, NM-121-25 and NM-54, respectively showed a positive relationship of disease severity with average relative humidity. Rainfall also showed positive relationship and highly significant with disease severity of *Cercospora* leaf spot. The mean values for all the four varieties of mung bean (NM-28, Kabuli mung, NM-121-25, NM-54) were 0.9265, 0.9455, 0.8911 and 0.9442, respectively. The mean values 0.5884, 0.6354, 0.5089, and 0.6249 for NM-28, Kabuli mung, NM-121-25 and NM-54 respectively showed a positive relationship of disease severity with wind speed. NM-28, Kabuli mung and NM-54 had a significant relationship although NM-121-25 had a non-significant relationship with disease severity (Table 2).

Effect of different treatments on disease severity of Cercospora leaf spot

All the treatments were effective against *Cercospora* leaf spot disease as compared to control. Boric acid (BA) showed (30.82%) severity, magnesium sulphate (MgS) showed (22.69%) severity, zinc sulphate (ZnS) showed (16.56%) of severity and combination of three chemicals named as, BA + ZnS + MgS showed (11.72%) of disease severity (Table 4).

Effect of treatments on growth parameters of mung bean

It was examined that vegetative growth disturbed by stress due to diseases which may limit the traits related to yield. At harvest stage height of the plant was measured with the help of measuring tape from top leaf of the mung bean plant to the region from where the plant stem started. Height of all infected plants was carefully measured and compared with control. As data was recorded different varieties showed different results when treated with different treatments as boric acid effect on average plant height of mung varieties was shown to be (42.23 cm) followed by magnesium sulphate (48.36 cm) and zinc sulphate showed (40.06 cm) and their combination boric acid + magnesium sulphate + zinc sulphate showed (55.86cm) as compared with the control (water) showed (33.20cm) plant height (Table 3).

After harvesting root length of the plant was measured with the help of measuring tape at the end point of stem of the mung bean plant to the tips of the root. Root length of all infected plants was carefully measured and compared with control. As data was recorded different varieties showed different

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results when treated with different treatments as boric acid effect on root length of mung was shown to be (21.93cm) followed by magnesium sulphate (24.83cm) and zinc sulphate showed (20.56cm) and their combination magnesium sulphate + zinc sulphate + boric acidshowed (28.76cm) as compared with the control (water) showed (16.13cm) root length.

Table 3: *Effect of treatments on plant growth parameters.*

Parameters	Control	BA	MgS	ZnS	BA+ MgS+ ZnS
Plant height (cm)	32.20 e	42.23 c	48.36 b	40.06 d	55.86 a
Root length (cm)	16.13 e	21.93 c	24.83 b	20.56 d	28.76 a
Fresh weight (g)	69.51 e	87.47 c	91.23 b	78.54 d	105.41 a
Dry weight (g)	6.40 e	8.37 ab	8.90 a	8.13 ab	9.88 a

Alpha = 0.05; Tukey HSD value = 4.3290

Table 4: *Effect of treatments on disease severity in all varieties.*

Treatment	Mean
BA	30.82 b
MgS	22.69 c
MgS+ZnS+BA	11.72 e
ZnS	16.56 d
Control	43.88 a
Alpha = 0.05	
Tukey HSD value = 3.5360	

Fresh weight of the plant was measured with the help of weighing balance in grams and the results revealed that different treatments have different effect on varieties of mung bean. The fresh weight of the plant is compared with the control as the results showed that the boric acid effect on fresh weight of mung was shown to be (87.47g) followed by magnesium sulphate (91.23g) and zinc sulphate showed (78.54) and their combination magnesium sulphate + zinc sulphate + boric acid showed (105.41g) as compared with the control (water) showed (69.51g).

Dry weight of the mungbean plant was measured in grams with the help of weighing balance and compared with different treatments of different varieties and results revealed different treatments have different effects on different varieties of mung bean. Effect of dry weight of the plant is contrast with the control as the results reveal that the boric acid effect on dry weight of mung was shown to be (8.37g) followed by magnesium sulphate (8.90g) and zinc sulphate showed (8.13g) and their combination boric acid + magnesium sulphate + zinc sulphate showed (9.88g) as compared with the control (water) showed (6.40g).

Mung bean is a short duration legume crop that is grown worldwide and known for its edible seeds and sprouts (Hassan *et al*., 2017). It is significant for its high nutritional content as well as fixes nitrogen and improves soil quality (Schreinemachers *et al*., 2020). Its yield is limited by many biotic and abiotic factors but the most important one is *Cercospora* leaf spot which is attributed by the production of cercosporin toxin (Abass *et al*., 2020). The use of resistant germplasm is the most promising way for the management of this disease. There was very trace level of durable resistance in mung bean germplasm against CLS disease under open field conditions and only a single variety LGG 460 regarded as up to the mark out of 200 entries (Das *et al*., 2019). The resistant germplasm could be incorporated into the breeding programs for the development of mung bean varieties having resistance to CLS with desirable characters that would be the long-term disease management (Kumar *et al*., 2020). In current study, none of the variety showed immune or highly resistant reaction against the CLS while 2 were highly resistant. These results are in accordance with that of (Iqbal *et al*., 2009) who found 30 accessions with resistant reaction against CLS while rest fell in moderately resistant to moderately susceptible categories. Zhimo *et al*. (2013) evaluated more than 50 entries in field conditions against artificial inoculation of *Cercospora* inoculum and found only 5 varieties with different level of resistance. CLS disease is a serious devastating factor for mung bean that indirectly threatens the food security by causing more than 90% yield losses in severe conditions (Bhat *et al*., 2014). The pathogen infection deteriorates the chloroplast cells of the leaf thus affecting the frequency and quantity of photosynthesis badly (Zeng, 2017).

Environmental circumstances have an impact on mung bean production and growth as described that disease, environment and their interaction all have an impact on productivity and quality of crop (Dencic *et al*., 2011). The effects of environmental aspects on plant like high and low temperature, relative humidity, wind speed and rainfall definitely have a positive or negative impact on disease and both pathogens and plants (Vela´squez *et al*., 2018). In current experiment, the effect of temperature on disease development was negative while disease severity increased positively with relative humidity, rainfall and wind speed. Several epidemiological studies have found that weather variables i.e., maximum and minimum temperatures, relative humidity, wind velocity, and rainfall (RF) have a significant impact on disease transmission. As temperature increases, wind speed and precipitation facilitate the transmission of inoculums, disease epidemics are accelerated (Woods *et al*., 2012). Humidity has also been identified as the most potential element for disease development, particularly during wet seasons (Huber and Gillespie, 1992). *C. canescenes* severity was increased with increase in moisture and decrease in temperature (Dimkpa *et al*., 2020).

The role of micronutrients in decreasing disease severity can be described to their participation in plant physiology and biochemistry, as there are several mechanisms that might influence a plant's response to infections (Stott and Secor, 2018). It was observed form the results that the combination of boric acid, zinc sulphate and magnesium sulphate showed minimum disease severity against *Cercospora* leaf spot disease. B suppressed *Verticillium* wilt symptoms in tomato plants, and there was no vascular darkening in the roots of B-supplied plants. This shows that B prevented xylem pathogen invasion (Farooq, 2012). Zinc enhances plant growth and anti-pathogenic activities of the plant (Afzal and Singh, 2023). The pathogen *C. canescens* reportedly decreases the seed germination and it has been found in many field experiments that $ZnSO_4$ application increases the seed germination (Das *et al*., 2019). CLS causes severe defoliation and subsequently less photosynthesis and plant growth leading towards reduced yield and poor quality of the produce. Foliar application of zinc and boron enhances plant growth and yield of the produce (Maliha *et al*., 2022). Plants that are unable to absorb enough sulfur will develop a yellowing of the leaves that resembles nitrogen shortage. The older leaves at the base of the plant are the first to be impacted by nitrogen deficiency, with the effect spreading upwards. Sulfur can be captured by gypsum deposits in the soil layers and older plants with extensive roots may be able to recover once they reach this level of soil (Bonnie, 2021).

The world scenario of climate change demands for devising the integrated and sustainable disease management strategies and mitigating the causes for climate change. The management of disease through foliar application of nutrients is a durable and multipurpose management strategy. There is dire need to expand these findings for implication on a large commercial scale.

Conclusions and Recommendations

Commonly, plant diseases caused by fungi are managed by using different fungicides, which have many drawbacks, like development of resistance; environmental hazards; a driving force behind climate change factors. The current study was focused upon the management of leaf spot disease by using different nutrients that is a sustainable and environmentally safe approach. It was observed that apart from disease management, growth and yield attributes of the plant were also enhanced.

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Novelty Statement

This is the very first study on epidemiology and management of leaf spot of mungbean in Pakistan. The disease was managed by unique combination of nutrients rather than traditional insecticides.

Author's Contribution

Shaista Ilyas: Conducted Trial.
Safdar Ali: Supervised Research.
Amer Habib: Co-supervised the trial.
Misbah Ali: Helped in data recording.
Muhammad Ahmad Zeshan: Technical assistance for write up.
Yasir Iftikhar: Proof reading.
Muhammad Usman Ghani: Statistical analysis.
Muhammad Umair: Helped in lab work.

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

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