LR-4809
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RESEARCH ARTICLE Legume Research- An International Journal, Volume Issue : ()

Management Strategies using Phytoextracts and Fungicides Against Cercospora Leaf Spot of Mungbean Incited by *Cercospora canescens* under *in vitro* and *in vivo* Conditions

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ABSTRACT

Background: Cercospora leaf spot of mungbean [*Vigna radiata* (L.)] caused by *Cercospora canescens.* It has become a severe menace to the growers of Rajasthan in India and in general causes economic losses under changing climatic scenario. Depending upon the temperature and humidity, it spreads rapidly in susceptible varieties causing premature defoliation and reduction in size of pods and grains. In this study management was taken through botanicals and fungicides is also an important segment in the present era, in view of hazards caused by toxic chemicals or being developed resistance in pathogens to fungicides and effective means for disease control.

Methods: In this field-laboratory investigation during *kharif* 2018 and *kharif* 2019 at Agronomy Farm, Sri Karan Narendra Agriculture University, Jobner, Jaipur, Rajasthan. The experiment was conducted for seven plant extracts at 5, 10 and 15 per cent concentrations were evaluated *in vitro* by poisoned food technique. In field experiments, further these fungicides applied as different concentrations *viz.,* 0.1, 0.2 and 0.3% and plant extracts (at 10% conc.) were assessed by two foliar applications to control the disease.

Result: Experiment was carried out in laboratory and field condition. In Plant extracts, the 100 per cent inhibition of mycelial growth was obtained with garlic extract at 10% concentration. In field conditions, two foliar applications of Hexaconazole (0.1%) were proved the most effective in reducing disease intensity (55.58%) and in increasing crop yield (56.50%) followed by Hexaconazole + Captan. In plant extracts, garlic extract (10%) proved superior in reducing disease intensity (52.85%) and in increasing crop yield (33.96%) followed by neem leaf extract.

Key words: *Cercospora canescens*, Cercospora leaf spot, Fungicide, Mungbean, Plant extracts.

INTRODUCTION

Mungbean [*Vigna radiata* (L.)] is one of the important pulse crop of India. It is widely cultivated throughout the Asia including India, Pakistan, Bangladesh, Sri Lanka, Thailand, Cambodia, Vietnam, Indonesia, Malaysia and South China. Leaf spot disease caused by *Cercospora canescens* Ellis and Martin is a serious disease in the mungbean growing areas of the country where high humidity prevails during the growing season. The productivity of pulse crops became stagnant for the last three decades because of less success in developing improved varieties and moreover it is grown on marginal and sub marginal lands. Average yield of the crop is very low mainly due to low inherent yield potential and susceptibility of the crop to diseases (Thakur *et al.* 1977). Unique ability of biological nitrogen fixation, deep root system, mobilization of insoluble soil nutrients and bringing qualitative changes in soil physical properties, make them known as soil fertility restorers (Masood *et al.* 2002). Among the diseases, Cercospora leaf spot is a serious disease of mungbean (Verma and Sandhu 1992). It is widely distributed all over the country where mungbean is cultivated.

The causal organisms of this disease are *Cercospora cruenta*, *C. canescens*. Among these, *Cercospora cruenta* is the most prevalent species (Talukder 1974). The disease starts appearing about 30 to 40 days after planting. Depending upon the temperature and humidity, it spreads rapidly in susceptible varieties causing premature defoliation and reduction in size of pods and grains (Grewal *et al.* 1980). Several workers had reported the effective control of the disease with the application of fungicides (Singh and Singh 1978). Among various factors responsible for low yields, biotic and abiotic stresses take a heavy toll of the crop, out of which diseases cause an estimated yield loss of 21.93 to 68.77 per cent (Sharma *et al.* 2008).

Disease management through botanicals and fungicides is also an important segment in the present era, in view of hazards caused by toxic chemicals or being developed resistance in pathogens to fungicides and effective means for disease control. In lieu of this, seven

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How to cite this article: Kumar, N., Singh, M., Lakhran, L., Prajapati, S. and Maurya, S. (2022). Management Strategies using Phytoextracts and Fungicides Against Cercospora Leaf Spot of Mungbean Incited by *Cercospora canescens* under *in vitro* and *in vivo* Conditions. Legume Research. DOI: 10.18805/LR-4809.

Submitted: 07-10-2021 **Accepted:** 18-08-2022 **Online:** 10-11-2022

phyto-extracts and seven fungicides were evaluated for two consecutive years (2018 and 2019) in field at Jobner, Jaipur (Rajasthan) under artificial epiphytotic conditions against Cercospora leaf spot of mungbean.

MATERIALS AND METHODS

Experimental site

The experiment was carried out during *kharif* 2018 and *kharif* 2019 at Agronomy Farm, Sri Karan Narendra Agriculture University, Jobner, Jaipur Rajasthan. Jobner is situated at latitude 26°5′N, longitude of 75°20′E and altitude of 427 meters above MSL (mean sea level). The region falls under semi-arid eastern plain (Agro Climatic Zone-III A) of Rajasthan, India.

Effect of plant extracts on mycelial growth inhibition of *Cercospora canescens* (*in vitro*)

The present investigation was carried out to evaluate the extracts of different plants species to know the possible presence of fungi toxicant properties against *C. canescens.*

Preparations of plant extracts

Taken 100 g of onion bulb (*Allium cepa*), garlic clove (*Allium sativum*), ginger rhizome (*Zingiber officinale*), leaves of neem (*Azadirachta indica*), datura leaves (*Datura stramonium*), tulsi leaves (*Ocimum tenuiflorum*) and alovera leaves (*Aloe barbadensis*) were collected from Department of Horticulture, S.K.N College of Agriculture, Jobner, Jaipur. For preparing plant extracts of plant parts including leaves and bulbs to be tested were first washed with tap water followed by sterilized water and then air dried. Weighed plant material was crushed in warring blender using 1:1 w/v amount of distilled water using 100 g of leaves and bulbs, separately and filtered through double layered muslin cloth. This was considered as 100 per cent concentration and used for dilution to make needed dilution. From these botanicals 5, 10 and 15 per cent concentration were made by making dilutions with distilled water.

The botanicals were added aseptically to standardize Malt Extract Agar so as to get desired concentrations. Twenty ml of poisoned medium was poured in each sterilized petriplates, suitable control were maintained without addition of botanicals. Five mm of fifteen days old fungal disc was taken from the periphery of the culture and was placed in the center of the poisoned medium of poured petriplates and incubated at 25±1°C for 15 days. The experiment was conducted in completely randomized design (CRD) with three replications.The diameter of the colony was recorded after fifteen days of inoculation by using Vernier caliper scale and per cent inhibition of mycelial growth was calculated by using following formula given by Vincent (1947) and data were statistically analyzed.

$$\text{Per cent growth inhibition} = \frac{C - T}{T} \times 100$$

Where,

C = Diameter of the colony in check (average of both diagonals).

T = Diameter of the colony in treatment (average of both diagonals).

Efficacy of plant extract against Cercospora leaf spot of mungbean under field conditions

For the management of Cercospora leaf spot of mungbean under field condition, the plant extracts mentioned under *in vitro* study were applied for foliar spray under natural field conditions. Experiment was conducted in the field during *kharif* 2018 and 2019. The seeds of susceptible variety samrat were sown in plots of 2 × 2.1 m^2 and replicated three times.

For preparing plant extracts of plant parts including leaves and bulbs to be tested were first washed with tap water followed by sterilized water and then air dried. Weighed plant material was crushed in warring blender using 1:1 w/v, amount of distilled water using 100 g of leaves and bulbs, separately and filtered through double layered muslin cloth. This was considered as 100 per cent concentration and used for dilution to make required dilution. The plant extracts were diluted with water in 10 per cent concentration separately and sprayed on plants. Two foliar applications of all the plant extracts were applied and started just after disease initiation in the field and second spray was given after 15 days of first spray. The disease intensity shown in Table 1 was recorded as per 0-9 rating scale as given by (Metha and Mondal 1978) and per cent disease intensity (PDI) was calculated using the formula of Prasad *et al*. (1979) and Uddin *et al*. (2013). The seed yield was recorded in q/ha.

Per cent disease intensity =

$$\frac{\text{Sum of total rating}}{\text{Total number of observation} \times \text{Highest grade in the scale}} \times 100$$

The per cent disease control (PDC) over control was calculated as

Per cent disease intensity =

$$\frac{\text{PDI in control - PDI in treatment}}{\text{PDI in control}} \times 100$$

Efficacy of fungicides against Cercospora leaf spot of mungbean under field conditions

For the management of Cercospora leaf spot disease of mungbean under field condition, the fungicides mentioned under in vitro study were used for foliar spray under natural field conditions during *kharif* 2018 and 2019. The seeds of susceptible variety Samrat were sown in RBD design with plots size of 2 × 2.1 m^2 and replicated three times, though methodology was similar, as mentioned under *in vivo* study of plant extract, except concentration of spray fungicides. The per cent disease intensity recorded after fifteen days of last spray of fungicides.

RESULTS AND DISCUSSION

The results obtained during the present investigation in respect of per cent mycelial growth inhibition, disease

intensity and yield attributes of mungbean are presented below.

Effect of plant extracts on mycelial growth inhibition of *Cercospora canescens* (*in vitro*)

Seven plant extracts were evaluated at three concentrations in the laboratory for their efficacy against *C. canescens* through Poisoned Food Technique. The data are presented in Table 2 and Fig 1 revealed that among the seven plant extracts evaluated, *Allium sativum* at 15, 10 and 5 per cent concentration was found best in inhibiting the mycelial growth of *C. canescens* (100%, 100% and 86.50%) and found significantly superior over all the other plant extracts, followed by *Azadirachta indica* (95, 90.10 and 75%), *Datura stramonium* (78, 65 and 56%), *Zingiber offcinale* (48, 42 and 35%) and *Ocimum tenuiflorum* (31.22, 28.32 and 26.44) at 15, 10, 5 per cent concentration, respectively. No inhibition on mycelial growth of *C. canescens* was recorded in *Allium cepa* and *Aloe barbadensis* at 5, 10 and 15 per cent concentration, respectively. The fungicidal spectrum of garlic has been reported by Singh *et al*. (2014) observed that the maximum percentage inhibition of mycelial growth was obtained from garlic at 10.0 and 15.0 per cent, followed by Datura and Neem at the same concentrations, on *Cercospora canescens*. Venturoso *et al*. (2011) found garlic clove extract was a good inhibitor of *C. Kikuchii* and also by Poornima *et al*. (2011) on *Cercospora beticola* and same result also reported by Kulkarni (2009) on *Cercospora truncatum.*

Table 1: Standards for the assessment of disease intensity (Metha and Mondal 1978).

Disease rating /grade	Per cent leaf area affected	Description
0	-	no infection
1	1-10	One spot to 10 per cent diseased area
3	11-30	11-30 per cent diseased area
5	31-50	31-50 per cent diseased area
7	51-70	51-70 per cent diseased area
9	>71	>71 per cent diseased area

Efficacy of plant extracts against Cercospora leaf spot of mungbean under field conditions

Efficacy of different plant extracts was carried out against Cercospora leaf spot of mungbean under natural field conditions. Results of pooled analysis (Table 3 and Fig 2) revealed that lowest disease intensity was recorded in Garlic clove extract with 24.61 per cent and 48.68 per cent decrease disease intensity over control. It was found significantly superior over all treatments, followed by Neem leaf extract (28.22%), Datura leaf extract (32.61%) and

Table 2: Effect of plant extracts on mycelial growth inhibition of *Cercospora canescens* (*In vitro*).

Plant extracts	Part used	Per cent inhibition of mycelial growth			Mean
		5%	10%	15%	
Azadirachta indica	Leaves	75.00	90.10	95.00	86.70
		(60.00)	(71.66)	(77.08)	(68.61)
Allium sativum	Clove	86.50	100.00	100.00	95.50
		(68.44)	(90.00)	(90.00)	(77.75)
Datura stramonium	Leaves	56.00	65.00	78.00	66.33
		(48.45)	(53.73)	(62.03)	(54.53)
Ocimum tenuiflorum	Leaves	26.44	28.32	31.22	28.66
		(30.94)	(32.15)	(33.97)	(32.37)
Allium cepa	Bulb	0.00	0.00	0.00	0.00
		(0.00)	(0.00)	(0.00)	(0.00)
Aloe barbadensis	Leaves	0.00	0.00	0.00	0.00
		(0.00)	(0.00)	(0.00)	(0.00)
Zingiber offcinale	Rhizomes	35.00	42.00	48.00	41.67
		(36.27)	(40.40)	(43.85)	(40.20)
Control	-	0.00	0.00	0.00	0.00
		(0.00)	(0.00)	(0.00)	(0.00)
Mean		34.87	40.68	44.03	
		(36.19)	(39.63)	(41.57)	
		S.Em ±	C.D (p=0.05%)	CV	
	P	0.43	1.19	5.27	
	C	0.70	1.95		
	P × C	1.21	3.37		

*Average of three replications.

Parenthesis are angular transformed value.

Ginger (34.59%) with 41.15, 32.00 and 27.86 per cent decrease intensity over control, respectively. Alovera leaf extract was found least effective with 44.94 per cent disease intensity and 5.34 decrease intensity over control followed by Onion bulb extract (43.50%) and Tulsi leaf extract (39.43%) with 9.29 and 17.78 per cent decrease intensity over control, respectively. Our results are in agreement with the result of Bdliya and Alkali (2010) on Cercospora leaf spot of groundnut and Uddin *et al.* (2013) on Cercospra leaf spot of mungbean. They found that Garlic clove extract and Neem leaf extract were found significantly superior to other plant extract. Sheshma *et al.* (2017) were evaluated efficacy of six plant extract against *Cercospora canesence* and found that Neem leaf extract, Garlic clove extract and Datura leaf extract were effective at 10 per cent concentration.

Efficacy of fungicides against Cercospora leaf spot of mungbean under field conditions

Seven different fungicides were tested for management of Cercospora leaf spot of mungbean by spraying twice at 15 days interval under field conditions. Two years pooled results on per cent disease intensity (Table 4 and Fig. 3) revealed that all the fungicides were found significantly effective in reducing the Cercospora leaf spot disease over control. The lowest disease intensity (22.07%) was recorded with the application of Hexaconazole with 55.58 per cent decreased disease intensity over control, which was statistically at par with application of Hexaconazole + Captan. However, Hexaconazole + Captan and Carbendazim + Mancozeb were found second and third best with 24.44, 29.05 per cent disease intensity with 50.81, 41.52 per cent decreased disease intensity, respectively. Copper oxychloride was

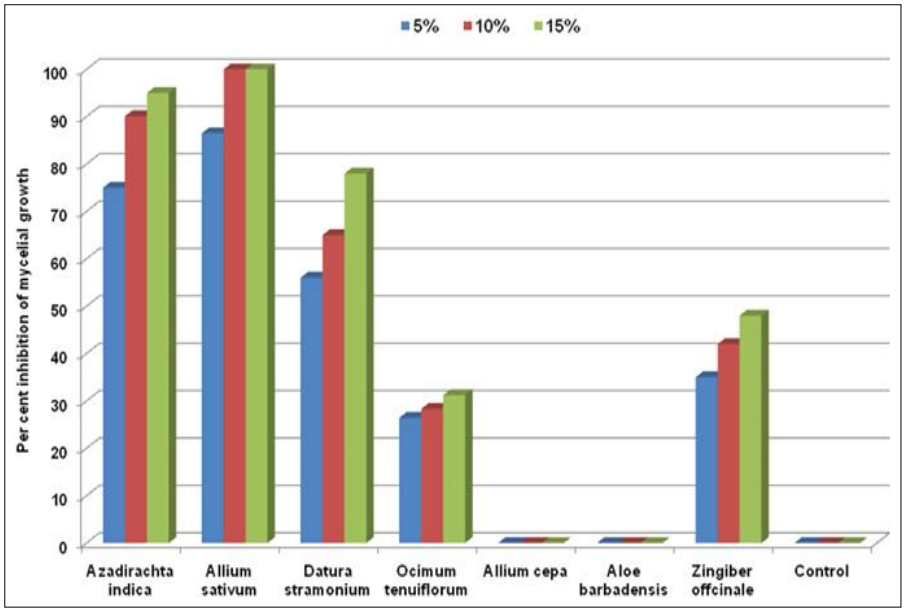

Fig 1: Effect of plant extracts on mycelial growth inhibition of *Cercospora canescens* (*In vitro*).

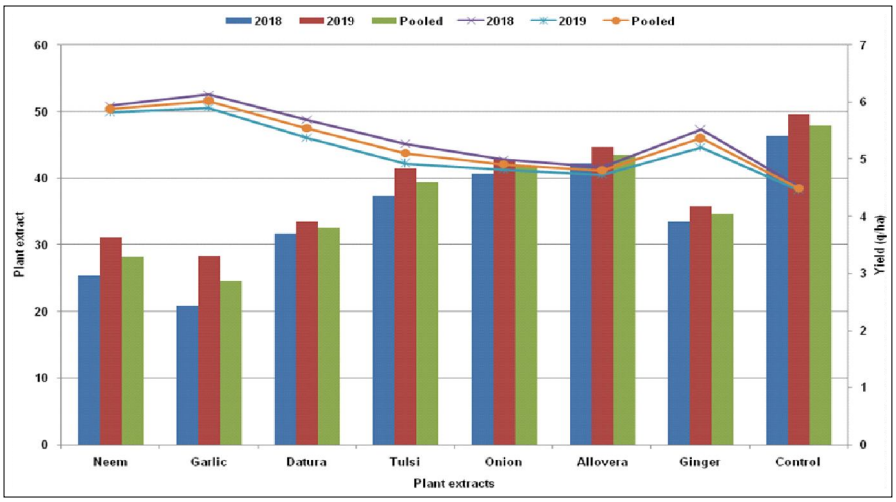

Fig 2: Efficacy of plant extract against Cercospora leaf spot of mungbean under field conditions.

Table 3: Response of foliar application of plant extracts on Cercospora leaf spot of mungbean and yield under artificial epiphytotic conditions.

Plant extracts	Conc. (%)	Plant extract			PDI over control	Yield (q/ha)			Increase in yield over control
		2018	2019	Pooled		2018	2019	Pooled	
Neem	10	25.33 (30.22)	31.11 (33.90)	28.22 (32.09)	41.15	5.93 (14.09)	5.83 (13.97)	5.88 (14.03)	30.96
Garlic	10	20.89 (27.20)	24.33 (29.55)	22.61 (28.39)	52.85	6.13 (14.33)	5.90 (14.06)	6.02 (14.20)	33.96
Datura	10	31.73 (34.28)	33.48 (35.35)	32.61 (34.82)	32.00	5.69 (13.80)	5.38 (13.41)	5.54 (13.61)	23.27
Tulsi	10	37.33 (37.66)	41.52 (40.12)	39.43 (38.89)	17.78	5.27 (13.27)	4.93 (12.83)	5.10 (13.05)	13.59
Onion	10	42.26 (40.55)	44.73 (41.97)	43.50 (41.26)	9.29	4.99 (12.91)	4.82 (12.68)	4.91 (12.80)	9.24
Aloevera	10	43.76 (41.42)	46.12 (42.77)	44.94 (42.10)	6.28	4.86 (12.74)	4.73 (12.56)	4.80 (12.65)	6.79
Ginger	10	33.42 (35.32)	35.76 (36.73)	34.59 (36.02)	27.86	5.52 (13.59)	5.21 (13.19)	5.37 (13.39)	19.49
Control	-	46.32 (42.89)	49.58 (44.76)	47.95 (43.83)	-	4.52 (12.27)	4.46 (12.19)	4.49 (12.23)	-
SEm±		1.47	1.58	1.52		0.13	0.15	0.17	
CD (P=0.05)		4.52	4.87	4.69		0.41	0.46	0.52	
CV		7.07	7.15	7.11		7.80	8.42	8.11	

*Average of three replications.

Parenthesis are angular transformed value.

Table 4: Efficacy of fungicides against Cercospora leaf spot of mungbean under field conditions.

Fungicides	Conc. (%)	Percent disease intensity			PDI over control	Yield (q/ha)			Increase in yield over control
		2018	2019	Pooled		2018	2019	Pooled	
Copper oxychloride	0.3	41.86 (40.32)	44.63 (41.9)	43.25 (41.12)	12.94	5.29 (13.30)	5.07 (13.01)	5.18 (13.16)	7.80
Hexaconazole	0.1	18.36 (25.37)	25.77 (30.5)	22.07 (28.02)	55.58	7.94 (16.37)	7.10 (15.45)	7.52 (15.92)	56.50
Propiconazole	0.2	31.76 (34.30)	33.28 (35.2)	32.52 (34.77)	34.53	6.37 (14.62)	6.22 (14.44)	6.30 (14.53)	31.01
Carbendazim	0.1	36.54 (37.19)	38.91 (38.5)	37.73 (37.89)	24.06	5.93 (14.09)	5.56 (13.64)	5.75 (13.87)	19.56
Thiophanate methyl	0.2	33.46 (35.34)	35.69 (36.6)	34.58 (36.02)	30.40	6.13 (14.33)	5.88 (14.03)	6.01 (14.18)	24.97
Hexaconazole + Captan	0.2	23.55 (29.03)	25.32 (30.2)	24.44 (29.62)	50.81	7.43 (15.82)	7.01 (15.35)	7.22 (15.59)	50.26
Carbendazim + Mancozeb	0.2	28.23 (32.09)	29.87 (33.1)	29.05 (32.61)	41.52	7.11 (15.46)	6.30 (14.54)	6.71 (15.01)	39.54
Control	-	48.92 (44.38)	50.43 (45.2)	49.68 (44.81)	-	4.96 (12.87)	4.65 (12.45)	4.81 (12.66)	0.00
SEm±		1.61	1.67	1.64		0.25	0.23	0.24	
CD (P=0.05)		4.96	5.15	5.06		0.77	0.71	0.73	
CV		8.02	7.95	7.99		7.57	7.53	7.55	

* Average of three replications.

Parenthesis are angular transformed value.

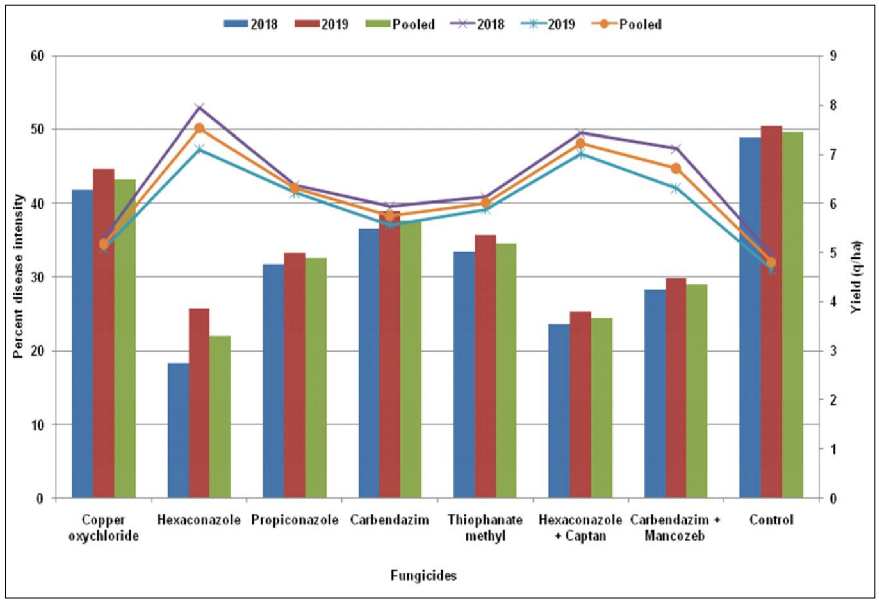

Fig 3: Efficacy of fungicides against cercospora leaf spot of mungbean under field condition.

found least effective with 43.25 per cent disease intensity and 12.94 per cent decreased disease intensity over control. These results are comparable with findings of Khunti *et al.* (2005), Sarkar *et al.* (2017) and Ganipurkar (2018) were reported that Hexaconozole effectively reduced the disease intensity with higher yield under *In vivo* condition.

CONCLUSION

It was observed that the cercospora leaf spot of mungbean caused heavy damage to grain yield. *In vivo* evalution of different fungicides and botanicals were tested, in which hexaconazole found most superior among all tested fungicides and gave maximum grain yield with minimum disease intensity. Among botanicals Garlic extract showed minimum disease intensity with maximum grain yield. Two foliar applications of *Allium sativum* (10%) at 15 days interval was also proved superior in reducing disease intensity and in increasing fruit yield followed by *Azadirachta indica*. Results of our experiments were worked on management of cercospora leaf spot of mungbean**.**

Conflict of interest: None.

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