In vitro and *In vivo* Evaluation of Fungicides, against *Colletotrichum lindemuthianum* causing Anthracnose of Mung Bean

A B S T R A C T

Mung bean [Vigna radiata (L.) Wilczek] is a popular pulse crop, second only to chickpea and pigeon pea, and is grown in many parts of the world bean and Mungbean bean belongs to family leguminaceae. In the suitable condition bean is attacked by various diseases. Out of which, anthracnose caused by seed borne pathogen *Colletotrichum lindemuthianum* is an important fungal disease and major limiting factor for yield loss. Realizing the potentiality of the disease in causing economic losses, the different fungicides are tested against *Colletotrichum lindemuthianum* among the fungicides Difenoconazole 25 %EC, Carbendazim50%WP, Metiram70%WG, Propiconazole25%EC, Pyraclostrobin20%WP, Propineb 70% WP, Carbendazim12%WP+ Mancozeb 63%WP, Hexaconazole 5% EC respectively

Keywords

Mung bean, *Colletotrichum lindemuthianum*, fungicides

Introduction

Mung bean [Vigna radiata (L.) Wilczek] is a popular pulse crop, second only to chickpea and pigeon pea, and is grown in many parts of the world, including East Asia, Southeast Asia, and the Indian subcontinent (Nair et al., 2012). Mung bean production is mostly (90 percent) concentrated in Asia; India is the greatest producer, accounting for more than half of global production but consuming nearly all of it. Mung beans are grown in significant quantities in China, accounting for 19% of total legume production. Thailand is the leading exporter, with annual production increasing by 22% between 1980 and 2000. (Lambrides et al., 2006)In 2014–2015, India produced 1.5–2.0 million tonnes of mungbean from 3–4 million hectares, with an average productivity of 0.5 t ha1 (Jadhav et al., 2016). It promotes soil fertility by fixing nitrogen from the atmosphere and is also utilized as a green manuring crop and livestock fodder. The mung bean, also known as green gram or mung bean, is a Kharif crop that belongs to the Fabaceae (Leguminousae) family. Mung Bean seeds, a rich source of protein, fibre, and iron, are used in the everyday diet of humans, particularly those living in poverty (AVRDC, 2012). Greengram sprouts and pods have a high concentration of essential vitamins and minerals (Keatinge et al., 2011). Mungbean is planted on 4.5 mha-1 in India, with a total production of 2.5 million tonnes of grains and a productivity of 5.48 q/ha expected in 2021. (Anonymous,2021) Green gram anthracnose was originally discovered in 1951 in Jorhat, Assam (Majid, 1953). Anthracnose, caused by *C.lindemuthianum* (Sacc. and Magn.) and affecting mung bean and other beans, has been identified as one of the most serious diseases, with potential yield losses in mung bean (Sharma et al. 2008; Padder et al.2010b; Jan et al.2014; Kulkarni and Benagi, 2015). Anthracnose-related yield losses have been estimated to be between 24 and 67 percent (Deeksha and Tripathi, 2002), with an 18.2 to 86.57 percent disease index reported in northern Karnataka (Laxman, 2006)

Materials and Methods

Isolation and pathogenicity

The present investigation on *in vitro* and *in vivo* efficacy of fungicides against *Colletotrichum lindemuthianum* was conducted various experiment will be conducted in research field, R.A.K.. College of Agriculture, Sehore (M.P.) at the Department of Plant Pathology, Post Graduate Institute,. The culture of *C. lindemuthianum* used in this study was isolated from infected leaves of Mung bean plants collected from the research field, R.A.K.. College of Agriculture, Sehore. In order to isolate pathogen infected leaf sample were cut along with healthy leaf and surface sterilized with 0.1% sodium hypochlorite solution for one minute and washing with three times by sterilized distilled water. The bits were placed in petriplates containing PDA medium. All the above operations were carried out in sterilized condition (under laminar air flow unit). Then plates were incubated at 27±2 ºC for 10 days. The fungal growth, which developed around each bit, was then transferred to PDA medium slant for sub culturing. The isolated fungi were identified as *C. lindemuthianum* on the basis of morphological characters and published literature. Pathogenicity of *C. lindemuthianum* in mungbean was proved by applying spray inoculation with spore and mycelial suspension of the pure culture of C. lindemuthianum on the seedlings of cultivar

In vitro evaluation of fungicides by poisoned food technique

Poisoned food technique was used to evaluate the efficiency of eight fungicides against pathogens. Potato dextrose agar medium was prepared and distributed at the rate of 100 ml in 250 ml conical flask, autoclaved 121 for 30 min. Then before solidification of media different fungicides with

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desired concentration were incorporated aseptically in different flasks. These flasks shaken to facilitate uniform mixture of fungicides thoroughly and poured in Petri plate's 20 ml/plate likewise three plates for each treatment were poured. One set of three plates was poured without any fungicides to serve as a control. After solidification of medium, the plates inoculated with seven days old pathogens separately. Five mm diameter mycelial disc selected from peripheral growth of the plate by sterilized cork borer were used for inoculating the plates by keeping one disc per plate in the centre in inverted position, so as to make the mycelial growth touch the surface of medium.

The inoculated plates were incubated at room temperature for seven days. The colony diameter of the fungal pathogens on medium was recorded and percent inhibition in each treatment was calculated by using following formula (Vincent, 1927).

$$\text{Percent inhibition} = \frac{C-T}{C} \times 100$$

Where,

PI = Per cent Inhibition

C = Growth of fungi in control (mm)

T = Growth of fungi in treatment (mm)

Results and Discussion

In vitro* evaluation of fungicides against *Colletotrichum lindemuthianum

Fungi toxic activities of different fungicides was assayed against *Colletotrichum lindemuthianum* and observed in (Table 1) indicated According to the study, all of the treatments were much more effective than the control at reducing *C.lindemuthianum* mycelial growth. in 250ppm Propiconazole 25% EC, Hexaconazole 5% EC, and Propineb 70% WP all showed minimal radial mycelial growth followed by all treatment. in 500 and 1000 ppm there is a no mycelia growth in Propiconazole 25% EC, Propineb 70% WP followed by Hexaconazole 5% EC, Difenoconazole 25 %EC, Carbendazim 50%WP, Carbendazim12%WP+ Mancozeb 63%WP, Metiram 70%WG and Pyraclostrobin 20%WP.

In vivo* evaluation of fungicides against *Colletotrichum lindemuthianum

The experiment was conducted during 2022, with eight treatments and one untreated control. sprays were given at the interval of seven days and next pre-tratment was observed after 7 day of spray in other concentration. The observation on the anthracnose development was recorded at seven days interval after spray.

Pre treatment

Before any spray of fungicides, anthracnose intensity was at par in all the treatments

After treatment

Minimum percent disease index was recorded in Propiconazole 25%EC in all the concentration followed by Hexaconazole 5% EC, Difenoconazole 25 %EC. followed by Propineb70%WP,Carbendazim12%WP+Mancozeb63%WP,Metiram70%WG,Carbendazim50%WP,Pyraclostrobin20%WP.

Maximum percent disease index was recorded in control (%). Apart from control maximum percent disease index was recorded from Pyraclostrobin 20%WP**.**

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Table 1 *In vitro* evaluation of fungicide

	Radial growth of *collectotrichum lindemuthianum* in fungicides amended medium at 250,500 and 1000PPM						
		Radial growth (mm)*			mycelium inhinition %		
S.N0.	Treatment	250ppm	500ppm	1000ppm	250ppm	500ppm	1000ppm
1	Difenoconazole 25 %EC	25.67	13.67	9.33	47.92	62.92	68.33
2	Carbendazim 50%WP	27.00	14.67	9.33	46.25	61.67	68.33
3	Metiram 70%WG	33.00	24.67	11.67	38.75	49.17	65.42
4	Propiconazole 25%EC	10.33	0.00	0.00	67.08	80.00	80.00
5	Pyraclostrobin 20%WP	34.67	29.67	15.67	36.67	42.92	60.42
6	Propineb 70% WP	12.00	4.00	4.00	65.00	75.00	75.00
7	Carbendazim12%WP+ Mancozeb 63%WP	28.67	16.33	12.33	44.17	59.58	64.58
8	Hexaconazole 5% EC	18.33	9.00	6.33	57.08	68.75	72.08
9	control	80.00	80.00	80.00			
	SE(m)±	1.474	1.625	0.667			
	C.D.at 5%	4.414	3.441	1.996			

*Average of three replications

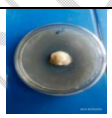
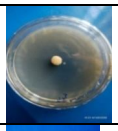
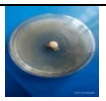
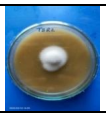
Tr. No.	250ppm	500ppm	1000ppm	Tr. No.	250ppm	500ppm	1000ppm
Difenoconazole 25 %EC				Propineb 70% WP			
Carbendazim 50%WP				Carbendazim12%WP+ Mancozeb 63%WP			
Metiram 70%WG				Hexaconazole 5% EC			
Propiconazole 25%EC				control			
Pyraclostrobin 20%WP							

Figure-1 Radial growth of *collectotrichum lindemuthianum* in fungicides amended medium at 250,500 and 1000PPM

Table-2 In vivo* evaluation of fungicides against *Colletotrichum Lindemuthianum.

		Percent disease index* of Anthracnose					
		250ppm		500ppm			1000ppm
S.NO.	Treatment	Pre - treatment	After spray	Pre - treatment	After spray	Pre - treatment	After spray
1	Difenoconazole 25 %EC	19.33	16.33 (23.77)	25.67	22.33 (28.19)	31.33	27.00 (31.29)
2	Carbendazim 50%WP	21.33	19.33 (26.05)	30.33	28.00 (31.94)	29.00	24.67 (29.77)
3	Metiram 70%WG	23.67	22.33 (28.19)	27.33	26.00 (30.65)	31.67	30.33 (33.41)
4	Propiconazole 25%EC	21.00	17.33 (24.57)	24.33	20.00 (26.55)	30.00	23.33 (28.87)
5	Pyraclostrobin 20%WP	23.33	22.33 (28.19)	27.67	26.67 (31.08)	32.67	31.33 (34.02)
6	Propineb 70% WP	21.00	19.67 (26.31)	25.33	24.00 (29.32)	32.00	30.00 (33.19)
7	Carbendazim12%WP+ Mancozeb 63%WP	22.33	20.00 (26.55)	26.00	23.33 (28.87)	29.33	26.33 (30.86)
8	Hexaconazole 5% EC	21.67	19.00 (25.83)	25.00	22.00 (27.96)	30.00	25.67 (30.41)
9	control	28.33	32.33 (34.63)	37.33	42.00 (40.38)	42.67	51.33 (45.75)
	SEm±	-	0.678	-	0.362	-	0.522
	C.D. at 5%	-	2.052	-	1.069	-	1.580

*Mean of three replications
() Data in parentheses are Angular transformed values

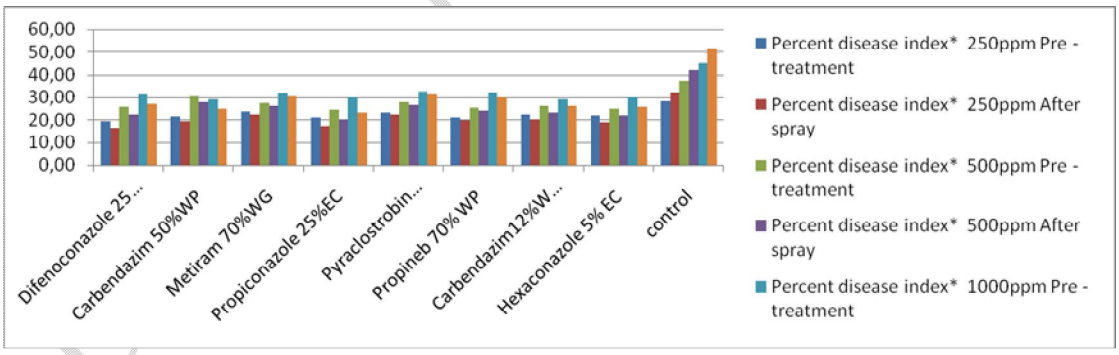


Fig. 2: Graphical presentation showing disease index with different treatment perspectives

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