



***In vitro* and *In vivo* Evaluation of Fungicides, against *Colletotrichum lindemuthianum* Causing Anthracnose of Mungbean**

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mungbean [*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. Mungbean belongs to family Leguminosae. 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, Carbendazim 50% WP, Metiram 70% WG, Propiconazole 25% EC, Pyraclostrobin 20% WP Propineb 70% WP, Carbendazim 12% WP+ Mancozeb 63% WP, Hexaconazole 5% EC respectively.

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1. INTRODUCTION

Mungbean [*Vigna radiate* (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 [1]. Mungbean production is mostly (90 %) concentrated in Asia; India is the greatest producer, accounting for more than half of global production but consuming nearly all of it. 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 per ha [2]. It promotes soil fertility by fixing nitrogen from the atmosphere and is also utilized as a green manuring crop and livestock fodder. The Mungbean, also known as green gram, is a Kharif crop that belongs to the Fabaceae (Leguminosae) family. Mungbean seeds, a rich source of protein, fibre, and iron, are used in the everyday diet of humans, particularly those living in poverty [3]. Greengram sprouts and pods have a high concentration of essential vitamins and minerals [4]. Mungbean is planted on 4.5 m ha in India, with a total production of 2.5 million tonnes of grains and a productivity of 5.48 q per ha expected in 2021 [5]. Green gram anthracnose was originally discovered in 1951 in Jorhat, Assam [6]. Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) and affecting Mungbean and other beans, has been identified as one of the most serious diseases, with potential yield losses in Mungbean [7-10]. Anthracnose-related yield losses have been estimated to be between 24 and 67 percent [11], with an 18.2 to 86.57 per cent disease index reported in northern Karnataka [12].

2. MATERIALS AND METHODS

The present investigation on in vitro and in vivo efficacy of fungicides against *Colletotrichum lindemuthianum* was conducted in research field of AICRP on MULLaRP, and Department of Plant Pathology, R.A.K. College of Agriculture, Sehore (M.P.). The culture of *Colletotrichum lindemuthianum* used in this study was isolated from infected leaves of Mungbean plants collected from the research field. 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°C 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 *Colletotrichum lindemuthianum* on the basis of morphological characters and published literature. Pathogenicity of *Colletotrichum lindemuthianum* in mungbean was proved by applying spray inoculation with spore and mycelial suspension of the pure culture of *Colletotrichum lindemuthianum* on the seedlings of cultivar.

2.1 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 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 [13].

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

Where,

PI = Per cent Inhibition

C = Growth of fungi in control (mm)

T = Growth of fungi in treatment (mm)

3. RESULTS AND DISCUSSION

3.1 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 *Colletotrichum lindemuthianum* mycelia growth. In 250 ppm 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, Carbendazim 12% WP+ Mancozeb 63% WP, Metiram 70% WG and Pyraclostrobin 20% WP.

3.2 In vivo Evaluation of Fungicides against *Colletotrichum lindemuthianum*

The experiment was conducted during 2022, with eight treatments and one untreated control. The observation on the anthracnose development was recorded at seven days interval after spray.

Table 1. In vitro evaluation of fungicide

Radial growth of <i>Colletotrichum lindemuthianum</i> in fungicides amended medium at 250,500 and 1000PPM							
S.NO.	Treatment	Radial growth (mm)*			Mycelium inhibition %		
		250 ppm	500 ppm	1000 ppm	250 ppm	500 ppm	1000 ppm
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	Carbendazim 12%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

Table 2. In vivo evaluation of fungicides against *Colletotrichum lindemuthianum*

S. no.	Treatment	Percent disease index*		
		Recommended Dose / liter	Pre - treatment	After spray
1	Difenoconazole 25 %EC	0.5 ml	19.33	16.33(23.77)
2	Carbendazim 50%WP	1 gm	21.33	19.33(26.05)
3	Metiram 70%WG	2 gm	23.67	22.33(28.19)
4	Propiconazole 25%EC	1 ml	21.00	17.33(24.57)
5	Pyraclostrobin 20%WP	2 gm	23.33	22.33(28.19)
6	Propineb 70% WP	2.5 gm	21.00	19.67(26.31)
7	Carbendazim 12%WP+ Mancozeb 63%WP	1 gm	22.33	20.00(26.55)
8	Hexaconazole 5% EC	1 ml	21.67	19.00(25.83)
9	Control		28.33	32.33(34.63)
	CD			0.678
	SE(m)			2.052

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

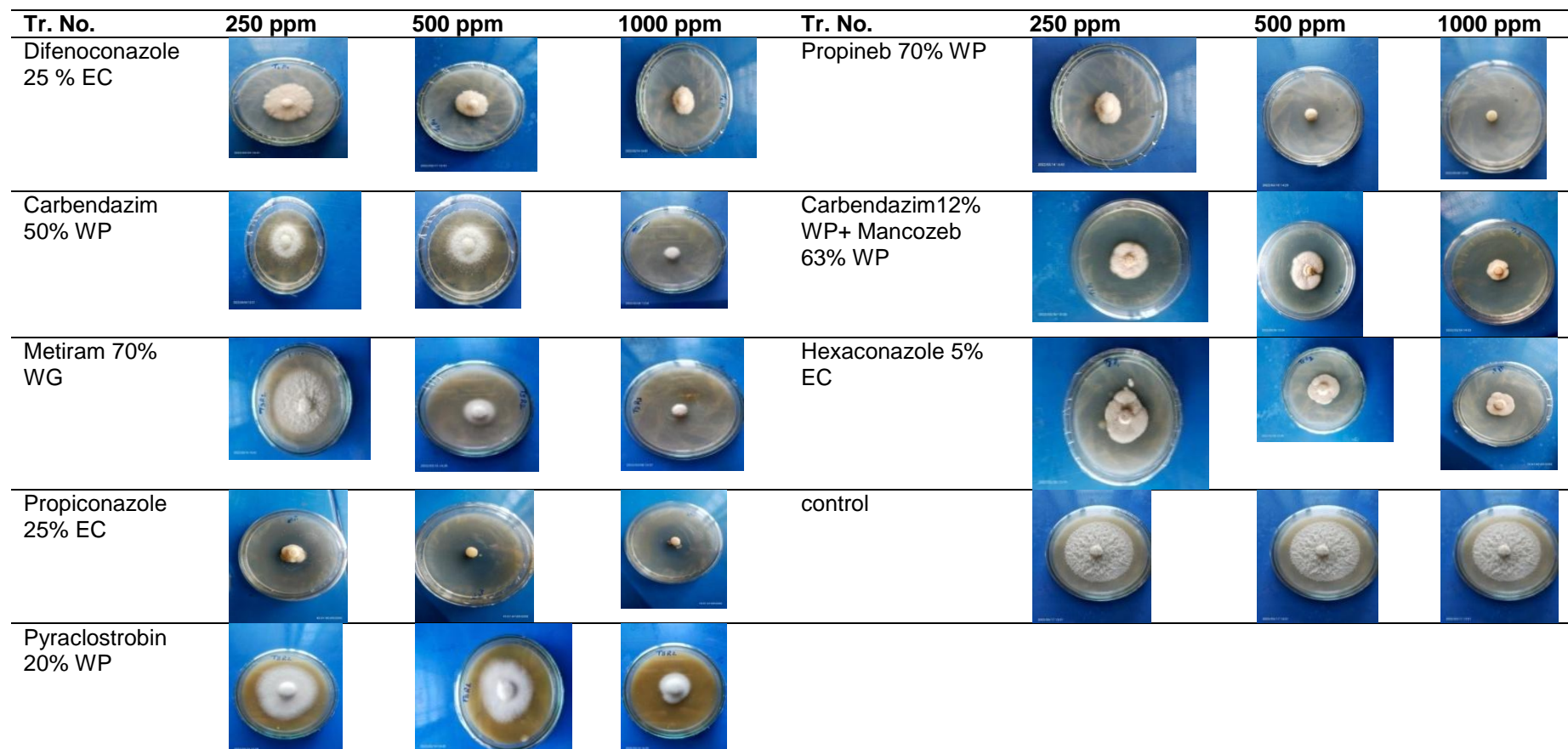


Fig. 1. Radial growth of *Colletotrichum lindemuthianum* in fungicides amended medium at 250,500 and 1000PPM

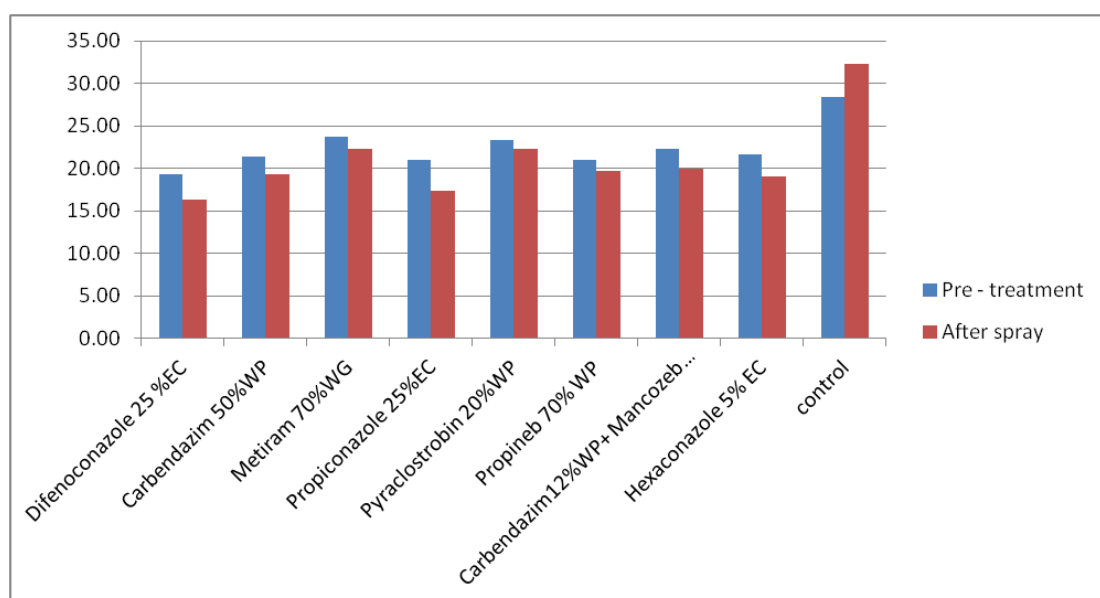


Fig. 2. Graphical presentation showing disease index with different treatment perspectives

3.2.1 Pre treatment

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

3.2.2 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 Propineb 70% WP, Carbendazim 12% WP+ Mancozeb 63% WP, Metiram 70% WG, Carbendazim 50% WP, Pyraclostrobin 20% WP.

Maximum per cent disease index was recorded in control. Apart from control maximum percent disease index was recorded from Pyraclostrobin 20% WP.

4. CONCLUSIONS

All the eight fungicides tested in vitro were found to inhibit mycelial growth of *C. lindemuthianum*. However, fungicide, Propiconazole 25% EC, was reported highly effective against mycelial growth inhibition of *C. lindemuthianum* as compared to all other treatments. In vivo fungicidal application of Propiconazole 25% EC (Foliar Application) was found most effective.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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