

Original Research Article

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## Evaluation of Fungicides against Target Leaf Spot Caused by (*Corynespora cassiicola*) *in vitro* Condition

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### ABSTRACT

#### Keywords

Leaf spot,  
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Target leaf spot disease of soybean caused by *Corynespora cassiicola*. The disease affects leaves, stems, pods and seeds. Leaf lesions are rounded to irregular and reddish brown; they vary from specks to big mature spots. Lesions are frequently surrounded by a dull green or yellowish green halo. In this study, *in vitro* evaluation of six fungicides at two concentrations (50 and 100 ppm) revealed that four fungicides *i.e.* Fluxapyroxad, Propiconazole, Tebuconazole and Hexaconazole completely inhibited the growth of the pathogen. Minimum inhibition of mycelial growth was recorded by Mancozeb and Pyraclostrobin in comparison to control at both (50 ppm and 100 ppm) the concentrations.

### Introduction

Soybean (*Glycine max.* L. Merrill) belonging to family Leguminaceae is designated as miracle bean established its potential as an industrially vital and viable oilseed crop in many areas of India. The target leaf spot disease of soybean causes by (*Corynespora cassiicola*) was first reported in 1945 (Olive *et al.*, 1945). Now it has been found in most of soybean growing states. In Chhattisgarh it has been reported during 2002 from Raipur (Patel, 2005). The disease affects leaves, stems, pods and seeds. Leaf lesions are rounded to irregular and reddish brown; they vary from specks to big mature spots. Lesions are frequently surrounded by a dull green or yellowish green halo. Severely affected leaves drop prematurely (Sinclair, 1982). The fungus

over winters on soybean debris and seed. It can survive in a fallow field for two years. The yield losses to an extent of 18-32 percent have been recorded in susceptible soybean lines grown in Mississippi during years when rainfall was above normal in August and September. Use of foliar fungicides has not been a reliable control method for target leaf spot. But few fungicides are available for management of target leaf spot of soybean. The most used MBC (methyl benzimidazole carbamate) fungicide is carbendazim (Ghini and Kimati, 2000). Jones and Jones (1985) tested 4 fungicides and reported mancozeb and chlorothalonil to be most effective against *C. cassiicola* under *in vitro* condition. Parakhia *et al.*, (1989) evaluated seven

fungicides under *in vitro*. Carbendazim and thiophanate-methyl were inhibitory to *C. cassiicola* at all concentration tested. There are numerous reports on fungicides *in vitro* condition to control the disease.

## Materials and Methods

### Experimental site

The laboratory experiment was carried out at Department of Plant Pathology, IGAU, and Raipur (C.G.)

### Isolation of test fungus

The fresh infected leaves of soybean plant samples were cut into small pieces, surface sterilized with 0.1% mercuric chloride (HgCl<sub>2</sub>) solution followed by three washing with sterile distilled water and placing in moist chamber than after 1 to 2 days fungal mycelium growth were seen than finally small bits of fungus kept on the previously poured and solidified potato dextrose agar medium in Petri plates for isolation of the pathogen. The plates were incubated at 25°C in BOD incubator.

### *In vitro* evaluation of fungicides

Poisoned food technique was employed for the evaluation of fungicides in the laboratory. Six fungicides viz. Pyraclostrobin, Fluxapyroxad, Propiconazole 25EC (Tilt), Mancozeb 75% WP (DithaneM-45), Tebuconazole 250 EC (Folicur), Hexaconazole 5 SC (Contaf). Were evaluated against *Corynespora cassiicola*. Two concentrations *i.e.*, 50 ppm, 100 ppm, of each treatment were used. The required quantity of fungicide was mixed with PDA at the time of pouring. Three replications were maintained for each fungicide for each of its concentration in CRD. The media was shaken well so as to enhance proper mixing of the

fungicides. To avoid bacterial contamination a little amount of streptomycin was added in each flask before plating; five mm disc was cut with the help of sterilized cork borer from seven days old culture of the test fungus and was placed in the center of the medium in the reversed position to maintain continuous contact of the pathogen with poisoned medium. PDA plates without fungicide served as control. The radial growth of the colony was measured when the growth in control plates reached the rim of the Petri plates. Percent growth inhibition under the influence of different fungicides was calculated on the basis of the control. Observation was recorded at 5 days, 10 days and 15 days after inoculation.

The per cent inhibition of radial growth, sporulation and spore germination over control was calculated as per the following formula (Vincent, 1977).

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

Where,

I = Inhibition percentage

C = Radial growth in control

## Results and Discussion

### Evaluation of fungicides against *C. cassiicola* under *in vitro* condition

Six fungicides [Pyraclostrobin, Fluxapyroxad, Propiconazole 25EC (Tilt), Mancozeb 75% WP (DithaneM-45), Tebuconazole 250 EC (Folicur), Hexaconazole 5 SC (Contaf)] were evaluated against mycelial growth of *C. cassiicola* to find out most effective fungicides. It was revealed from the data presented in the (Table 1 and Plate 1) that all the chemical fungicides significantly inhibited the growth of *C. cassiicola* at both the levels

of concentration *i.e.* 50 ppm and 100 ppm. At 100 ppm four fungicides *i.e.* Fluxapyroxad, Propiconazole, Tebuconazole and Hexaconazole completely inhibited the growth of the pathogen and were at par with each other. Minimum inhibition of mycelial growth was recorded by Mancozeb (30.59%) and Pyraclostrobin (75.37%) in comparison to control (Figure 1). On the basis of performance at 100 ppm all six fungicides

again evaluated at lower concentration (50 ppm) against *C. cassiicola*. Data presented in table 2 and figure 2 indicates that fungicides Fluxapyroxad, Propiconazole, Tebuconazole and Hexaconazole again completely inhibited the mycelial growth of the pathogen. Mancozeb and Pyraclostrobin inhibited the mycelial growth by 25.37% and 67.53% respectively over control.

**Table.1** Evaluation of fungicides at 100ppm concentration on mycelial growth of *C. cassiicola* at *in vitro* condition

S. No	Treatments	5 DAI		10 DAI		15 DAI	
		Growth (mm)*	Inhibition (%)	Growth (mm) *	Inhibition (%)	Growth (mm) *	Inhibition (%)
1	Pyraclostrobin	0.00	100	14.00	82.42	22.00	75.37
2	Fluxapyroxad	0.00	100	0.00	100	0.00	100
3	Propiconazole	0.00	100	0.00	100	0.00	100
4	Mancozeb	13.33	72.41	40.00	49.78	62.00	30.59
5	Tebuconazole	0.00	100	0.00	100	0.00	100
6	Hexaconazole	0.00	100	0.00	100	0.00	100
7	Control	48.33	-	79.66	-	89.33	-
<b>SE(m)</b>		<b>0.41</b>		<b>0.66</b>		<b>0.82</b>	
<b>C.D. (5%)</b>		<b>1.28</b>		<b>2.04</b>		<b>2.53</b>	

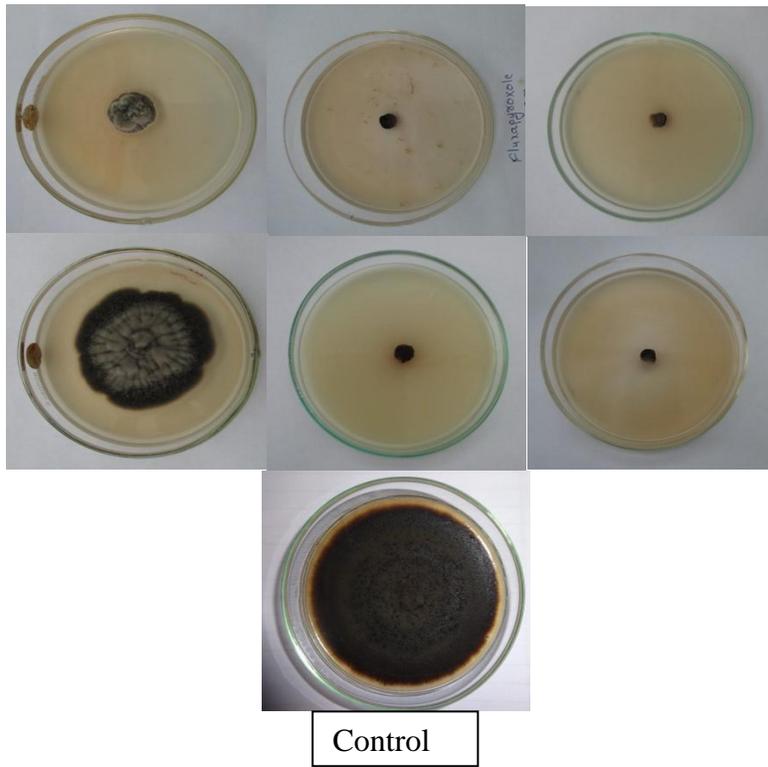
\*Average of three replication

**Table.2** Evaluation of fungicides at 50ppm concentration on mycelial growth of *C. cassiicola* at *in vitro* condition

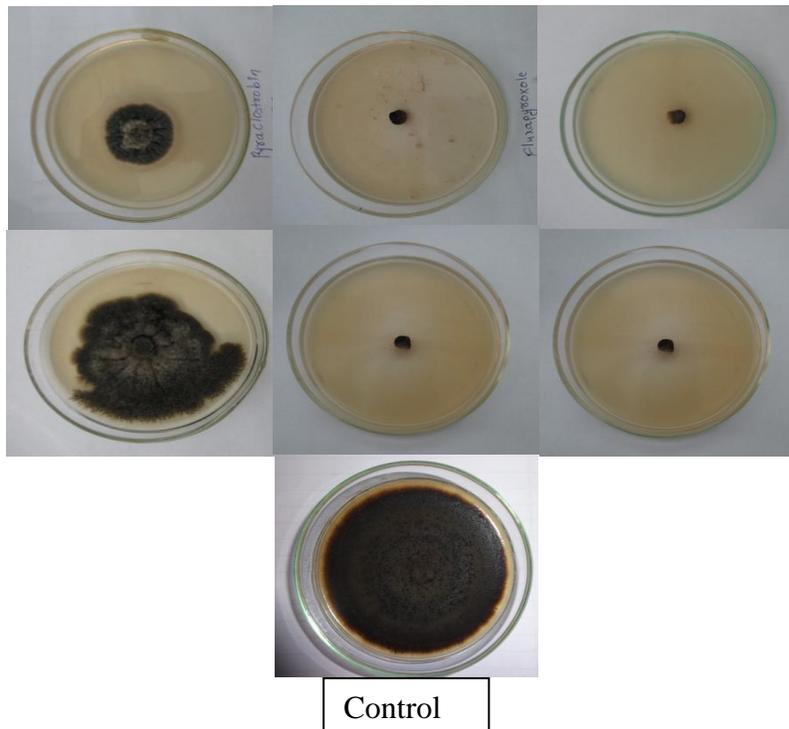
S. No	Treatments	5 DAI		10 DAI		15 DAI	
		Growth (mm) *	Inhibition (%)	Growth (mm) *	Inhibition (%)	Growth (mm) *	Inhibition (%)
1	Pyraclostrobin	8.33	82.76	19.33	75.73	29.00	67.53
2	Fluxapyroxad	0.00	100	0.00	100	0.00	100
3	Propiconazole	0.00	100	0.00	100	0.00	100
4	Mancozeb	19.66	59.32	51.00	35.97	66.66	25.37
5	Tebuconazole	0.00	100	0.00	100	0.00	100
6	Hexaconazole	0.00	100	0.00	100	0.00	100
7	Control	48.33	-	79.66	-	89.33	-
<b>SE(m)</b>		<b>0.65</b>	-	<b>1.30</b>	-	<b>1.35</b>	-
<b>C.D. (5%)</b>		<b>2.00</b>	-	<b>3.99</b>	-	<b>4.15</b>	-

\*Average of three replication

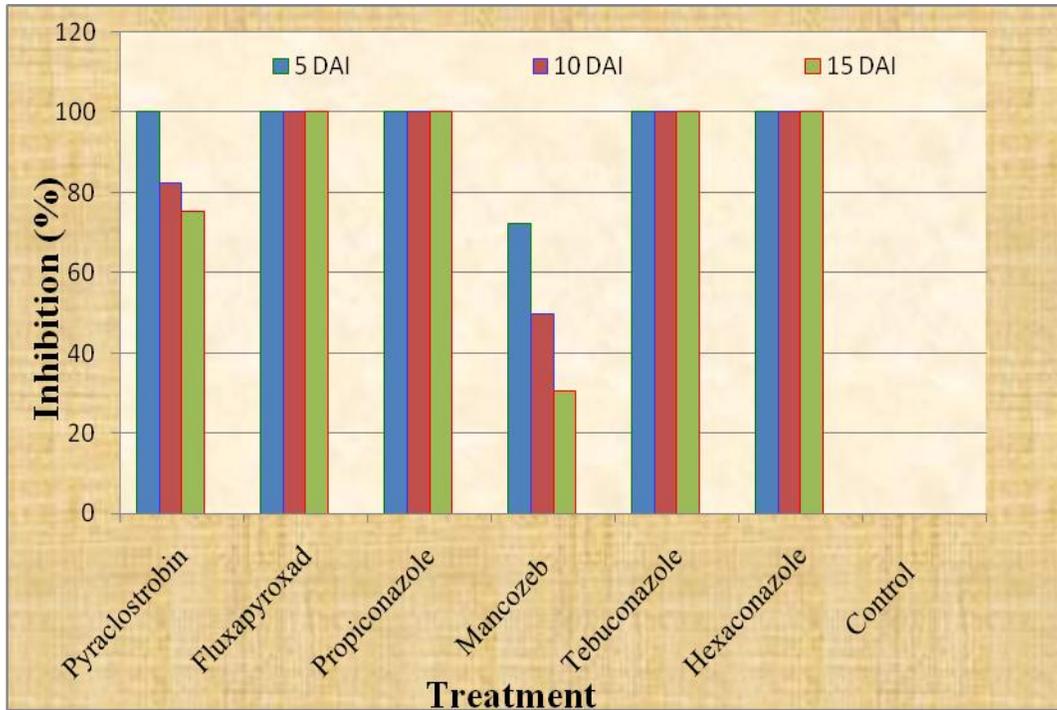
**Plate.1** Evaluation of fungicides at 100ppm concentration on mycelia growth of *C. cassiicola* at *in vitro* condition



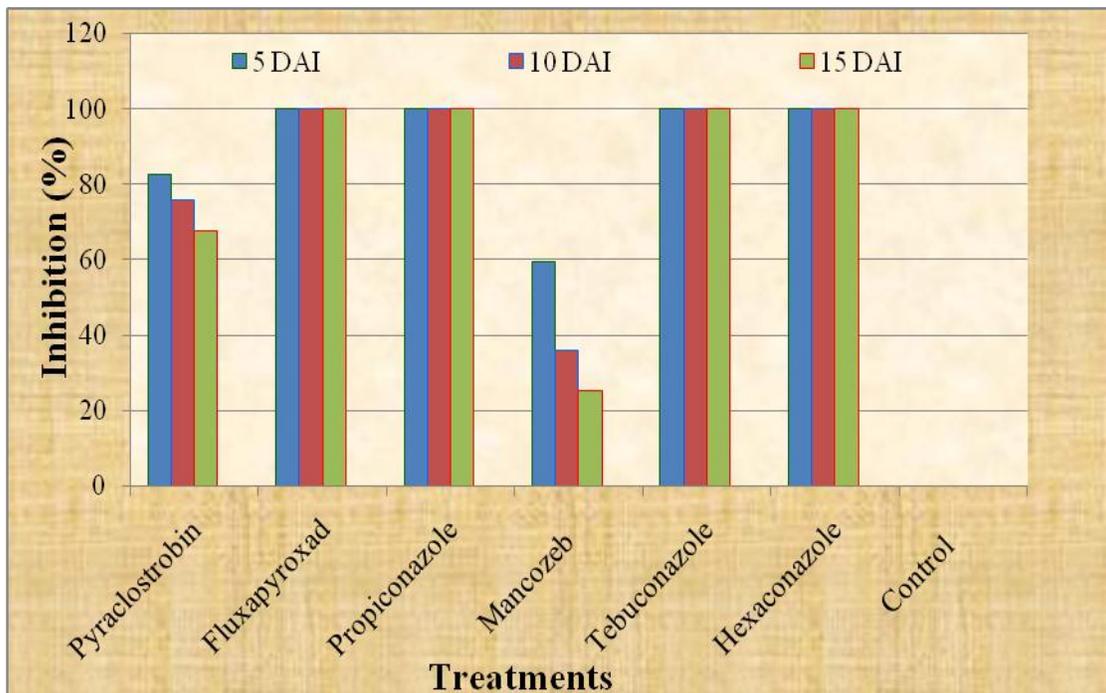
**Plate.2** Evaluation of fungicides at 50ppm concentration on mycelial growth of *C. cassiicola* at *in vitro* condition



**Fig.1** Evaluation of fungicides at 100ppm concentration on mycelial growth of *C. cassiicola* at *in vitro* condition



**Fig.2** Evaluation of fungicides at 50ppm concentration on mycelial growth of *C. cassiicola* at *in vitro* condition



These results are in confirmation with Parakhia *et al.*, (1989) evaluated seven fungicides under *in vitro*. Carbendazim and thiophanate-methyl were inhibitory to *C. cassiicola* at all concentration tested. Jones and Jones (1985) tested four fungicides and reported most effective against *C. cassiicola* under *in vitro* condition.

Xavier *et al.*, (2013), studied the incidence of target spot disease on soybean has increased in recent years in Brazil even with intensive use of fungicides, and fungal resistance has been reported in recent studies. They determine the fungicide sensitivity to carbendazim and prothioconazole in a sample of 24 isolates of *C. cassiicola* from soybean collected from 1996 to 2011 in the states of Parana Mato Grosso and Sao Paulo (Brazil) and Corpus Christi (Paraguay). The 50% effective concentration (EC50) values were estimated by the relative mycelium growth reduction on fungicide-amended medium with the doses of 0, 0.5, 1, 10, 100 and 1000µg of active ingredient/ml.

The present study in evaluation of fungicides against *C. cassiicola* under *in vitro* condition result showed Fluxapyroxad, Propiconazole, Tebuconazole and Hexaconazole were very effective in two (50 ppm and 100 ppm) concentrations in reducing mycelia growth of *C. cassiicola* under lab conditions.

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