

Original Research Article

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## ***Invitro* Evaluation of Strobilurin and Triazole Fungicides against Frogeye Leaf Spot caused by (*Cercospora sojina* Hara.)**

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Frogeye leaf spot (FLS), caused by *Cercospora sojina* Hara, is a vital disease of soybean [*Glycine max* (L.) Merrill] in most soybean growing countries of the world. Lesions of frogeye leaf spot on leaves begin as small, gray, water-soaked spots and develop into gray to brown spots surrounded by narrow, dark reddish-brown margins. These spots were circular to angular in shape and vary in size. As the lesions age, the central areas become ash gray to light brown. *In vitro* evaluation of seven fungicides viz., tebuconazole, prochloraz + tebuconazole, azoxystrobin + tebuconazole + prochloraz, pyroclostrobin + mefentrifluconazole, tebuconazole + sulphur, carbendazim + mancozeb and hexaconazole, completely inhibited the growth of *Cercospora sojina* at 50ppm concentration.

### **Introduction**

Soybean [*Glycine max* (L.) Merrill] is a legume crop and is the second largest after groundnut oilseed in India. It is growing in diverse agro-climatic conditions. Soybean ranks first among the oilseeds in the world and contributes for nearly 25% of the world's total oil and fats production. The USA leads in terms of area and production of soybean, while India ranks fourth in area and fifth in production in the world. USA, Argentina, Brazil, China and India are the major producers of soybean accounting for 90 percent of world production. Productivity of soybean in India (830 kg/ha) is less than

global (2800 kg/ha) average due to abiotic and biotic stresses (Anno.2019).

Symptoms of FLS are most visible and typically seen on leaves, but may also occur on stems, pods, and seeds late in the growing season with prolonged conditions that favor disease development (warm and humid conditions). Lesions of frogeye leaf spot on leaves begin as small, gray, water-soaked spots and develop into gray to brown spots surrounded by narrow, dark reddish-brown margins. These spots were circular to angular in shape and vary in size. As the lesions age, the central areas become ash gray to light brown with thin, reddish-brown margins. On

the underside of leaves, the spots are darker and have light-to dark-gray centres. (Phillips *et al.*, 1999)

Strobilurins are an important class of fungicides that come from the discovery of *Strobilurus tenacellus*, the mushroom fungus that causes wood-rotting. This isolated natural fungicide is thought to be used to protect the fungus against microbes in the decomposition of the wood. The discovery of strobilurins led scientist to isolate and produce synthetic strobilurins by chemically altering the compound to be able to tolerate sunlight (Vincelli, 2012).

Fungicides of triazoles group (difenoconazole, propiconazole, tebuconazole and bitertanol), dithiocarbamate (mancozeb), benzimidazole (carbendazim) and phthalimide (chlorothalonil) were evaluated *in vitro* against *Cercospora arachidicola* and *Cercospora personatum*, the causal agents of tikka disease of groundnut. Tebuconazole at 50ppm and carbendazim at 100ppm completely suppressed the germination of the spores of both the pathogens (Mushrif *et al.*, 2017).

## **Materials and Methods**

### **Experimental site**

The field experiments were conducted during *Kharif* 2018 at the experimental field of Department of Plant Pathology, R.A.K. College of Agriculture, Sehore (M.P.).

### **Isolation, Purification and Identification of pathogen**

Small pieces of infected tissue (2-3mm in length) frog-eye leaf spot were cut at the junction of diseased and healthy portion with the help of disinfected blade after surface sterilizing with alcohol. These bits were

surface sterilized in 0.1 per cent mercuric chloride solution (HgCl<sub>2</sub>) for 30 seconds followed by three washing with sterilized distilled water in Petri plates under aseptic conditions using laminar air flow. These bits were then dried by placing on sterilized blotting paper. Five bits were transferred aseptically to the sterile Petri plates containing potato dextrose agar (PDA) medium. Inoculated Petri plates were incubated at 25 ± 2°C for five to seven days and examined at frequent intervals to see the growth of the fungus/conidia developing from different pieces.

The appearing fungus (*Cercospora sojina*) was observed after 72 hours and isolations were made from developing colonies for further study. The pathogen was further purified by hyphal tip method and sub-cultured on PDA slants kept at 4 °C for further study (Dhingra and Sinclair, 1985). On the basis of morphological characters fungus causing frog-eye leaf spot disease of soybean was identified and Isolated as *Cercospora sojina* (Hara, 1915).

### ***In vitro* evaluation of fungicides**

The poison food technique (Nene and Thapliyal, 1979) was followed to evaluate the efficacy of fungicides in inhibiting the mycelial growth of *Cercospora sojina*. Strobilurin and Triazole fungicides used in the present investigation are one concentration i.e., 50ppm of each fungicides were used. Three replications were kept for each concentration. *Cercospora sojina* was grown on PDA medium for 15 days prior to setting up the experiment. The PDA medium was prepared and melted. Required quantity of fungicides was added to the melted medium to obtain the required concentration on the basis of active ingredient present in the chemical. Little amount of streptomycin was added in each flask before plating to avoid

bacterial contamination. Twenty ml of poisoned medium was poured in each sterilized Petri plates. Suitable check was maintained without addition of fungicides. The plates were then inoculated as described earlier and incubated at 25 ±1 °C. The mycelium growth as colony diameters was measured after 3 days, 6 days and 9 days of inoculation. The inhibition percentage of each fungicide on *C. soja* was determined by using the formula given by Vincent (1947).

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

Where,

I = Per cent inhibition in growth of test pathogen

C = Radial growth (mm) in control

T = Radial growth (mm) in treatment

## Results and Discussion

### *In vitro* evaluation of Strobilurin and Triazole fungicides against caused by *Cercospora soja*

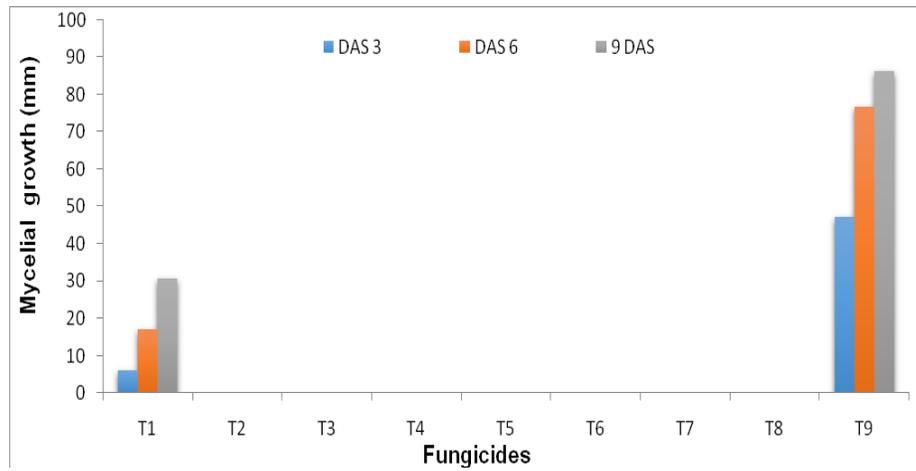
The data presented in the table 1 and fig.1 indicates that at 50ppm all the fungicides significantly inhibited the growth of *Cercospora soja* as compared in control (Plate- 1). Complete inhibition of *C. soja* was noticed at 50ppm in seven fungicides viz., tebuconazole, prochloraz + tebuconazole, azoxystrobin + tebuconazole + prochloraz, pyraclostrobin + mefentrifluconazole, tebuconazole + sulphur, carbendazim + mancozeb and hexaconazole. However, pyraclostrobin also inhibited 64.47% growth.

**Table.1** Radial growth of *Cercospora soja* on fungicides embedded medium at 50ppm concentration

S. No	Treatment	Mean radial growth (mm)*on			Final Inhibition (%)
		3 <sup>rd</sup> day	6 <sup>th</sup> day	9 <sup>th</sup> day	
1	Pyraclostrobin	6.00	17.00	30.66	64.47
2	Tebuconazole	0.00	0.00	0.00	100
3	Prochloraz + Tebuconazole	0.00	0.00	0.00	100
4	Azoxystrobin + Tebuconazole +Prochloraz	0.00	0.00	0.00	100
5	Pyraclostrobin + Mefentrifluconazole+ Fluxapyroxad	0.00	0.00	0.00	100
6	Tebuconazole + Sulphur	0.00	0.00	0.00	100
7	Carbendazim + Mancozeb	0.00	0.00	0.00	100
8	Hexaconazole	0.00	0.00	0.00	100
9	Control	47.33	76.66	86.33	–
	SE(m) ± 1	0.22	0.29	0.31	–
	CD at 5%	0.66	0.88	0.94	–

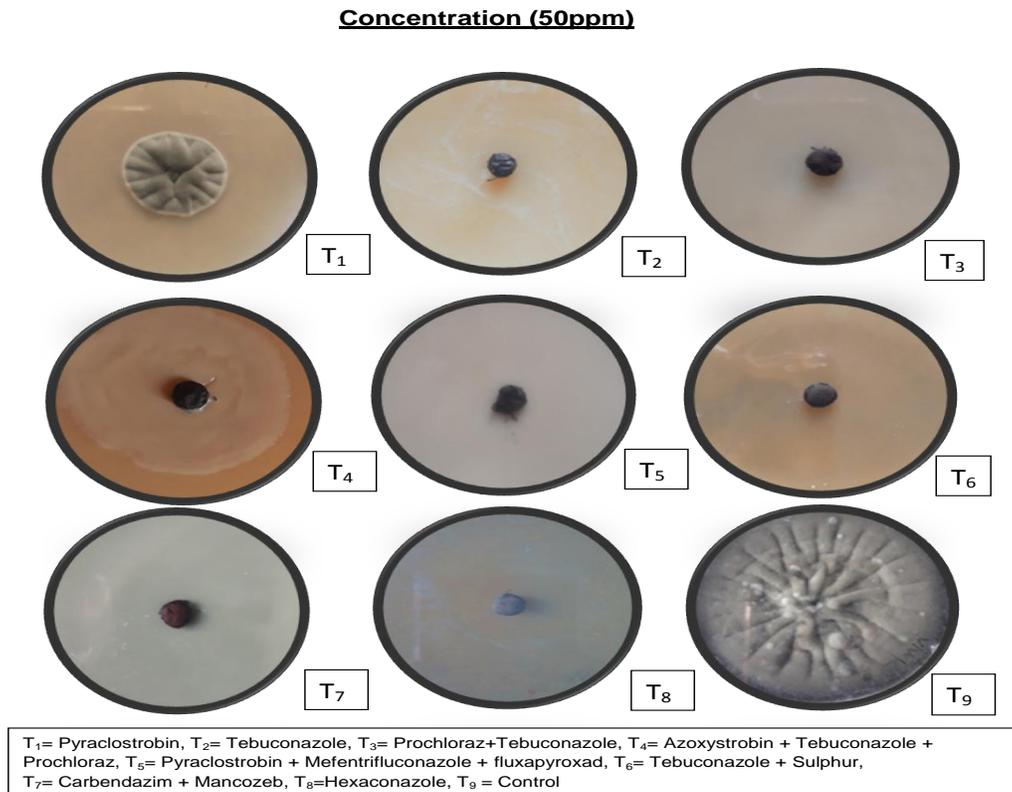
\*Average of three replications

**Fig.1** Mycelial growth of *Cercospora sojina* at 50ppm concentration of different fungicides



T<sub>1</sub> – Pyraclostrobin, T<sub>2</sub> – Tebuconazole, T<sub>3</sub> – Prochloraz + Tebuconazole, T<sub>4</sub> – Azoxystrobin + Tebuconazole + Prochloraz, T<sub>5</sub> – Pyraclostrobin + Mefentrifluconazole + fluxapyroxad, T<sub>6</sub> – Tebuconazole + Sulphur, T<sub>7</sub> – Carbendazim + Mancozeb, T<sub>8</sub> – Hexaconazole, T<sub>9</sub> – Control

**Plate.1** Mycelial growth of *Cercospora sojina* at 50ppm concentration of different fungicides



Mycelial growth of *C. soja* was found only in pyraclostrobin (6mm, 17mm and 30.66mm) amended media. Media without fungicide showed 47.33, 76.66 and 86.33mm growth.

The present study found complete inhibition of *C. soja* was noticed at 50ppm in seven fungicides viz., tebuconazole, prochloraz + tebuconazole, azoxystrobin + tebuconazole + prochloraz, pyraclostrobin + mefenftrifluconazole, tebuconazole + sulphur, carbendazim + mancozeb and hexaconazole. Therefore, future increased concentrations were not studied.

Prashanth, (2004) reported similar results of hexconazole and combo fungicides carbendazim 12% + mancozeb 63% for *C. kikuchii*. While Mushrif *et al.*, (2017) found complete suppression of spore germination of *Cercospora archidicola* by tubeconazole (50ppm) and carbendazim (100ppm).

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