**Original Research Article**

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

Vivek Kumar Vishwakarma¹*, Moly Saxena¹, Vijay Kumar Kashyap¹ and R. B. Jatav²

¹Department of Plant Pathology, RVSKVV College of Agriculture, Sehore (M.P), India
²Department of Plant Pathology, JNKVV, Jabalpur (M.P.), India

*Corresponding author

**A B S T R A C T**

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.

**Keywords**

Fungicides, Frogeye Leaf Spot, *Cercospora sojina*

---

**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 scientists 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).

In vitro evaluation of fungicides

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 ºC for further study (Dhingra and Sinclair, 1985). On the basis of morphological characters fungus causing frogeye leaf spot disease of soybean was identified and isolated as Cercospora sojina (Hara, 1915).

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) frogeye 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 (HgCl₂) 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.

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 ºC. 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. sojina 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 sojina**

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

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

| S. No | Treatment                                             | Mean radial growth (mm)*on | Final Inhibition (%) |
|-------|-------------------------------------------------------|----------------------------|----------------------|
|       |                                                       | 3rd day | 6th day | 9th 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

![Bar chart showing mycelial growth of Cercospora sojina at 50ppm concentration of different fungicides](image)

| Concentration (50ppm) |
|------------------------|
| Fungiicides            |

T₁ = Pyraclostrobin, T₂ = Tebuconazole, T₃ = Prochloraz + Tebuconazole, T₄ = Aoxystrobin + Tebuconazole + Prochloraz, T₅ = Pyraclostrobin + Mefentrifluconazole + fluxapyroxad, T₆ = Tebuconazole + Sulphur, T₇ = Carbendazim + Mancozeb, T₈ = Hexaconazole, T₉ = Control

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

![Images of mycelial growth at 50ppm concentration](image)
Mycelial growth of *C. sojina* 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. sojina* was noticed at 50ppm in seven fungicides viz., tebuconazole, prochloraz + tebuconazole, azoxystrobin + tebuconazole + prochloraz, pyroclostrobin + mefentrifluconazole, tebuconazole + sulphur, carbendazim + mancozeb and hexaconazole. Therefore, future increased concentrations were not studied.

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

References

Anonymous 2019. Online Agriculture Statics. Http/www.faostal.org.

Dhingra, O.D. and Sinclair, J.B. 1985. “Culture of Pathogens,” Basic Plant Pathology Methods, CRC Press, Boca Raton. pp: 11-47.

Hara, K. 1915. Spot disease of soybean. Agr. Country.9: 28.

Mushrif, S.K.; Manju, M.J.; Shankarappa, T.H. and Nagaraju 2017. Comparative efficacy of fungicides against tikka disease of groundnut caused by *Cercospora arachidicola* and *Cercosporidium personatum*. The ecosan 11(1&2): 62-71.

Nene, Y.L. and Thapliyal, P.N. 1979. Fungicides in plant disease control. Oxford and JBH Publishing Co., New Delhi. pp: 413.

Phillips, D.V. (1999). Frogeye leaf spot. p. 20–21. In G.L. Hartman, J.B. Sinclair, and J.C. Rupe (ed.) Compendium of soybean diseases. 4th ed. American Phytopathological Soc., St. Paul, MN.

Prashanth, S. P., 2004, Investigation on purple seed stain of soybean caused by *Cercospora kikuchii*. M. Sc. (Agri.) Thesis, Univ. Agric. Sci., Dharwad (India).

Vincelli, P. 2012. QoI (Strobilurin) Fungicides: Benefits and Risks. APS net Features. doi:10.1094/PHI-I-2002-0809-02.

Vincent, J. M. 1947. Distribution of fungal hyphae in per cent of certain inhibitors Nature, 96: 596.

How to cite this article:

Vivek Kumar Vishwakarma, Moly Saxena, Vijay Kumar Kashyap and Jatav, R. B. 2021. *In vitro* Evaluation of Strobilurin and Triazole Fungicides against Frogeye Leaf Spot caused by (*Cercospora sojina* Hara.). *Int.J.Curr.Microbiol.App.Sci.* 10(01): 794-798. doi: https://doi.org/10.20546/ijcmas.2021.1001.097