***In Vitro* Evaluation of Fungicidal Efficacy Against *Sclerotium rolfsii* in Soybean**

**ABSTRACT**

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| **Aim:** To assess the efficacy of newly approved and recommended fungicides in controlling collar rot disease of soybean under *in vitro* conditions.  **Study design:** *In-vitro* assessment of fungicide efficacy and statistical analysis of mycelial inhibition percentage of collar rot fungus.  **Place of Study:** Division of Crop Protection, ICAR-National Institute of Soybean Research, Khandwa Road, Indore.  **Methodology:** Infected plant samples were collected to isolate collar rot fungi and cultured on Potato Dextrose Agar (PDA). The *Sclerotium rolfsii* isolate displayed white, cottony mycelium and brown sclerotia (Approximately 0.32 mm). Sclerotia were transferred to PDA plates acidified with 0.2% lactic acid to establish a pure culture, with weekly sub-culturing for maintenance of the pathogen *S. rolfsii*. Fungicide efficacy was assessed by adopting poison food technique. PDA plates were amended with fungicides registered in India, including foliar fungicides (picoxystrobin, hexaconazole, pyraclostrobin + epoxiconazole, kresoxim-methyl, propiconazole, picoxystrobin + propiconazole, tebuconazole) at 0.05%, 0.1%, 0.15%, and 0.2%, and seed treatment fungicides (thiophanate methyl + pyraclostrobin, penflufen + trifloxystrobin, carboxin + thiram, carbendazim + mancozeb) at four different concentrations *viz*., 0.025%, 0.05%, 0.1%, and 0.125%. A Mycelial disc measuring 4 mm from a 4-day-old culture was placed in the centre of poisoned PDA plates, incubated at 26 ± 1°C with a 14h light/10h dark cycle. Mycelial growth inhibition was calculated using Vincent’s formula.  **Results:** The analysis showed significant differences in growth inhibition among fungicides (P < 0.0001), concentrations (P < 0.0001), and their interaction (P < 0.0001) for foliar applications, but no significant differences for seed dressing fungicides. In foliar applications, a 0.125% concentration (72.39%) was more effective than lower concentrations, with tebuconazole (100.00%) showing the highest inhibition of *S. rolfsii*. For seed dressing, all fungicides at all concentrations completely suppressed mycelial growth (100.00%) of *S. rolfsii*.  **Conclusion:** Tebuconazole effectively controlled *S. rolfsii* as foliar applications, while seed treatments with thiophanate methyl + pyraclostrobin, penflufen + trifloxystrobin, carboxin + thiram, and carbendazim + mancozeb completely inhibited mycelial growth. Further field studies are to be conducted to confirm their practical efficacy in managing collar rot in soybean. |

***Keywords:*** *Soybean, fungicide, collar rot, foliar, seed treatment.*

**1. INTRODUCTION**

Soybean (Glycine max (L.) Merrill), commonly known as the "golden bean," is an important leguminous crop valued for its high protein content (31.9%–45%) and edible oil concentration (14%–21.7%) (Singh *et al*., 2021a). Soybean cultivation worldwide, including in India, has consistently encountered significant challenges due to various diseases (Allen *et al*., 2017; Wrather *et al*., 2010).

In India, soybean cultivation is affected by several diseases, including anthracnose, charcoal rot, Rhizoctonia root rot, rust, yellow mosaic disease, and collar rot (Nataraj *et al*., 2019; Rajput *et al*., 2024; Rajput *et al*., 2023 and Dalal *et al*., 2022). Collar rot, caused by the fungal pathogen Sclerotium rolfsii, presents a serious risk, particularly within the prevalent soybean-chickpea (Cicer arietinum L.) cropping system in Central India (Ramteke *et al*., 2024). This cropping pattern heightens the vulnerability to soil-borne diseases such as collar rot, resulting in yield losses ranging from 30% to 40% (Sharma *et al*., 2014), which can escalate to 65% under severe conditions (Agarwal & Kotasthane, 1971). High soil moisture levels further exacerbate the problem, causing seedling mortality rates of up to 65% (Gupta & Nair, 2015). In 2006, S. rolfsii was responsible for an estimated 156.9 thousand metric tons of soybean yield loss in India, highlighting its growing impact on production (Wrather *et al*., 2010). Effective management strategies are crucial to safeguard soybean production from this destructive pathogen (Rajput *et al*., 2025a).

Collar Rot of Soybean (CRS) is widespread throughout India and tends to be more severe in low-lying areas with waterlogged soil conditions (Gupta & Chauhan, 2005). S. rolfsii is a necrotrophic, soil-borne phytopathogen responsible for causing damping-off during both pre- and post-emergence stages of soybean. Collar rot of soybean (CRS) is characterized by initial water-soaked, light brown lesions at the collar region of infected seedlings. As the disease advances, these lesions lead to tissue decay, resulting in seedling wilting and drooping. A distinctive symptom of CRS includes the formation of cottony white mycelium around the collar region, accompanied by the development of numerous mustard-sized sclerotial bodies on the infected soybean plants (Gupta & Chauhan, 2005). S. rolfsii sclerotia can survive in the soil for long durations in a dormant state, withstanding extreme temperatures, both heat and cold. Germination is often triggered by volatile compounds like methanol released by plants, with the pathogen using plant tissues as a nutrient source to support further infection and disease progression (Beute & Rodriguez-Kabana, 1979). As a necrotrophic pathogen, Sclerotium rolfsii destroys plant tissues by secreting various hydrolytic enzymes and toxins that facilitate infection and disease development (Gupta & Chauhan, 2005). The complex mode of action employed to establish pathogenesis in host plants, combined with the pathogen's robust survival strategies, makes the management of this pathogen particularly challenging (Ramteke *et al*., 2024).

Soilborne diseases negatively impact plant growth, lower grain quality, and reduce marketable yields in soybean cultivation. Since fully resistant cultivars are lacking for many of these diseases, management largely relies on fungicide applications and agronomic practices (Wen *et al*., 2017).

The fundamental principle of disease management is the cultivation of healthy and clean seeds (Rajput *et al*., 2020). However, producing such seeds can be challenging due to the presence of seed-borne fungi that often remain asymptomatic (Singh *et al*., 2021b). Seed treatment and foliar application with an effective fungicide offers a cost-effective and efficient method for controlling plant diseases (**Kumar *et al*., 2024**). Limited researchers have reported the effective management of CRS diseases through the use of fungicides (Rahman *et al*., 2020; Rahman *et al*., 2024).

After the government-imposed restrictions on specific fungicides, only limited officially approved fungicides are available to soybean growers in India. Which Includes picoxystrobin, hexaconazole, pyraclostrobin + epoxiconaxole, kresoxim-methyl, propiconazole, picoxystrobin + propiconazole, tebuconazole, thiophanate methyl + pyraclostrobin, penflufen + trifloxystrobin, carboxin + thiram, and carbendazim + mancozeb. In this context, the present study was conducted to assess the effectiveness of newly approved and recommended fungicides in controlling CRS in *in vitro* condition with controlled environmental conditions.

**2. MATERIALS AND METHODS**

**2.1 Collection and identification of fungal pathogen:**

The pathogen responsible for collar rot was isolated from an infected soybean plant collected from the plant pathology field at ICAR-National Institute of Soybean Research, Khandwa Road, Indore, following the methodology outlined in an earlier study by Dalal *et al*., 2022. Small sections (5–6 mm) of the infected stem were surface sterilized using a 0.1% sodium hypochlorite solution for 45 seconds, then rinsed with sterile distilled water, and placed on potato dextrose agar (PDA) supplemented with 100 ppm streptomycin. The *S. rolfsii* isolate exhibited white, cottony mycelia with a white colony appearance and produced brown-colored sclerotia measuring approximately 0.32 mm in size (Hartman *et al*., 2015). Sclerotia of *S. rolfsii* were visually identified and collected from Petri plates, then transferred onto PDA plates acidified with 0.2% lactic acid to develop a pure culture. Pure fungal cultures were transferred onto potato dextrose agar (PDA) slants and incubated at 28 ± 2°C for 15 days. The primary culture slants were stored at 5°C for up to one month for future use. To maintain the cultures, sub-culturing was carried out on a weekly basis.

**2.2 *In- Vitro* evaluation of different fungicides against collar rot**

The fungicides were evaluated with the help of the poison food technique to identify the most effective seed dresser fungicides under lab conditions (Rajput *et al*., 2016). PDA plates were amended with fungicides registered in India to evaluate their efficacy. The tested registered fungicides for foliar application included picoxystrobin (Galileo, Corteva Agriscience, India), hexaconazole (Contaf, Rallis India Limited, India), pyraclostrobin + epoxiconazole (Opera, BASF, India), kresoxim-methyl (Ergon, Rallis India Limited, India), propiconazole (Tilt, Syngenta India Ltd., India), picoxystrobin + propiconazole (Galileo Way, Corteva Agriscience, India), and tebuconazole (Folicur, BAYER India), applied at concentrations of 0.05%, 0.1%, 0.15%, and 0.2%. Additionally, combination fungicides registered for seed treatment, thiophanate methyl + pyraclostrobin (Xelora, BASF India Ltd.), penflufen + trifloxystrobin (EverGOL Xtend, Bayer India), carboxin + thiram (Vitavax Power, Dhanuka Agritech Ltd.), and carbendazim + mancozeb (Sprint, Indofil Industries Ltd., India), were tested at concentrations of 0.025%, 0.05%, 0.1%, and 0.125%. A 4 mm mycelial disc, taken from the periphery of a four-day-old culture, was placed at the centre of Petri plates containing poisoned PDA. The poisoned PDA containing Petri plates was incubated at 26 ± 1°C with 14 h and 10 h cycle of light/dark in BOD incubator. After full growth in control Petri plates, we calculated the mean mycelia growth inhibition percentage using formula I =100(C-T) /C**,** Where, I was mycelium growth inhibition per cent, C and T were mycelia growth in control and treatment (Vincent's formula (1947)). Three replicates were maintained for each treatment. The colony diameter was measured in two perpendicular directions, and the average was calculated. Data Analysis was carried out with analysis of variance (ANOVA) and least significant difference (LSD) test by using the R package “agricolae” (Rajput *et al*., 2025b)

**3. RESULTS AND DISCUSSION:**

**3.1 *In-vitro* evaluation of fungicides registered for foliar application against collar rot**

The pooled analysis of the mean growth inhibition percentage revealed significant differences among fungicides (P < 0.0001), concentrations (P < 0.0001), and their interaction (fungicide × concentration) (P < 0.0001) (Table 1). These results indicate that the mean growth inhibition percentage of *S. rolfsii* varied significantly across the different fungicides, the tested concentrations, and the combined effect of fungicides and their respective concentrations.

Mean growth inhibition percentage of *S. rolfsii* was significant higher with concentration 0.125% (72.39%) compared to 0.1% (64.06 %), 0.05% (59.88 %) and 0.025 % (54.28 %). Previous studies have also reported that higher concentrations of fungicides (0.1% and above) effectively inhibit the mycelial growth of S. rolfsii (Mishra *et al*., 2020; Kapadiya and Moradiya, 2017; Khan and Javaid, 2015).

Among the fungicides tested tebuconazole (100.00 %) showed significantly higher mean growth inhibition percentage of *S. rolfsii*, followed by pyraclostrobin + epoxiconaxole (87.00 %), compared to other evaluated fungicides. The picoxystrobin failed to inhibit the mean growth of *S. rolfsii*. Among the evaluated fungicides at different concentrations, tebuconazole exhibited complete inhibition (100%) of *S. rolfsii* at all tested concentrations, demonstrating significantly higher efficacy than the other fungicides.

Previous studies have demonstrated similar findings, where Mishra *et al*., (2020) reported complete inhibition of S. rolfsii mycelial growth in lentil using tebuconazole at concentrations of 0.03%, 0.05%, and 0.1%. Similarly, Kapadiya and Moradiya (2017) identified tebuconazole as the most effective fungicide for managing collar rot in groundnut with different concentrations. Tebuconazole acts by inhibiting 14α-demethylase (CYP51), an essential enzyme in the cytochrome P450-dependent oxidative demethylation of 24-methylene-24,25-dihydrolanosterol, a key precursor in ergosterol biosynthesis. Ergosterol plays a vital role in maintaining membrane fluidity and structural integrity (Rodrigues, 2018; Shcherbakova *et al*., 2021).

**Table 1: Percent inhibition of mean mycelial growth (%) of registered fungicides for foliar application against *S. rolfsii***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Foliar application Fungicides** | **Concentration (%)** | | | | **Mean** |
| **0.025** | **0.05** | **0.1** | **0.125** |
| Picoxystrobin | 0.00l | 0.00l | 0.00l | 0.00l | 0.00g |
| Hexaconazole | 71.82f | 73.94f | 81.69de | 86.78c | 78.56d |
| Pyraclostrobin + Epoxiconaxole | 80.84e | 84.31cd | 91.43b | 91.43b | 87.00b |
| Kresoxim-methyl | 0.00l | 17.98k | 23.92j | 63.92gh | 26.46f |
| Propiconazole | 60.90i | 62.94hi | 66.27g | 74.04f | 66.04e |
| Picoxystrobin + Propiconazole | 66.39g | 80.00e | 85.10c | 90.59b | 80.52c |
| Tebuconazole | 100.00a | 100.00a | 100.00a | 100.00a | 100.00a |
| Mean | 54.28d | 59.88c | 64.06b | 72.39a |  |

The data presented are the mean values obtained from four replicates per treatment. Statistical differences were determined using ANOVA followed by LSD test at p < 0.05. Treatments sharing the same superscript letter are not significantly different, while different superscript letters indicate significant differences at p < 0.05.

**3.2 *In-vitro* evaluation of fungicides registered for seed treatment application against collar rot**

The pooled analysis of the mean growth inhibition percentage didn’t reveal significant differences among fungicides (P > 0.05), concentrations (P > 0.05), and their interaction (fungicide × concentration) (P > 0.05) (Table 1). These results indicate that the mean growth inhibition percentage of *S. rolfsii* didn’t vary significantly across the different fungicides, the tested concentrations, and the combined effect of fungicides and their respective concentrations.Four seed dressing fungicides were evaluated with four different concentrations, 0.025 %, 0.05 %, 0.1% and 0.125%, against *S. rolfsii.* Interestingly,all fungicides at each concentration completely suppressed (100.00 %) the mean mycelia growth of the phytopathogens *S. rolfsii* (Table 2). Previous researchers also reported that it completely inhibited mycelia growth of *S. rolfsii* through pyraclostrobin (Amule *et al*. 2014; Najera *et al*. 2018), thiophanate methyl (Khan and Javaid 2015), carbendazim + mancozeb, carboxin + thiram (Shirsole *et al*. 2019). The complete suppression of pathogens is linked to the distinct mechanisms of action of the fungicides tested. Trifloxystrobin and pyraclostrobin, classified under the strobilurin/QoI group, disrupt mitochondrial respiration by binding to the quinol oxidation site of cytochrome b. This blocks the electron transfer from cytochrome b to cytochrome c1, halting energy production and inhibiting pathogen growth (Feng *et al*., 2020). Penflufen and carboxin function as succinate dehydrogenase inhibitors, interfering with the oxidative phosphorylation process and the tricarboxylic acid cycle, ultimately leading to energy deprivation in fungal cells (Di *et al*., 2021).

Benzimidazole fungicides, such as thiophanate methyl and carbendazim, hinder fungal cell division by blocking β-tubulin synthesis, which reduces spore formation and mycelial development. Meanwhile, dithiocarbamate fungicides like mancozeb and thiram exhibit multisite activity, disrupting key metal and sulfhydryl-containing enzymes necessary for fungal growth and reproduction (Yang *et al*., 2011).

**Table 2: Percent inhibition of mean mycelial growth (%) of registered fungicides for seed treatment application against *S. rolfsii***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Foliar application Fungicides** | **Concentration (%)** | | | | **Mean** |
| **0.025** | **0.05** | **0.1** | **0.125** |
| Thiophanate Methyl 450g/l + Pyraclostrobin 50g/l w/v FS | 100.00a | 100.00a | 100.00a | 100.00a | 100.00a |
| Penflufen 13.28% w/w + Trifloxystrobin 13.28% w/w FS | 100.00a | 100.00a | 100.00a | 100.00a | 100.00a |
| Carboxin37.5%+ Thiram37.5%DS | 100.00a | 100.00a | 100.00a | 100.00a | 100.00a |
| Carbendazim 25%+ Mancozeb 50% WS | 100.00a | 100.00a | 100.00a | 100.00a | 100.00a |
| Mean | 100.00a | 100.00a | 100.00a | 100.00a |  |

The data presented are the mean values obtained from four replicates per treatment. Statistical differences were determined using ANOVA followed by LSD test at p < 0.05. Treatments sharing the same superscript letter are not significantly different, while different superscript letters indicate significant differences at p < 0.05.

**4. CONCLUSION**

The current research aims to identify effective fungicides for managing collar rot disease in soybean. Among the seven registered fungicides tested for foliar application, tebuconazole exhibited the highest efficacy, significantly reducing the mean mycelial growth of *S. rolfsii* and surpassing all other treatments. For seed treatment, the combinations fungicide thiophanate methyl + pyraclostrobin, penflufen + trifloxystrobin, carboxin + thiram, and carbendazim + mancozeb were all equally effective, achieving complete inhibition of mycelial growth of the test fungus, S. rolfsii. These findings highlight the potential of both foliar and seed-treatment fungicides in effectively managing S. rolfsii in soybean cultivation. Additional field studies are required to validate these findings and evaluate the practical efficacy of these fungicides in managing collar rot in agricultural environments.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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