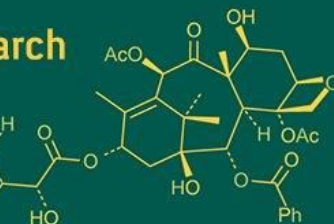
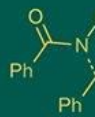


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In vitro suppression of major soil-borne pathogens

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Abstract

Aims: To evaluate the efficacy of six fungicides against three major soil-borne pathogens viz., *Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotium rolfsii* under *in vitro* conditions using the poisoned food technique.

Study Design: Completely randomized *in vitro* laboratory experiment

Place and Duration of Study: Department of Plant Pathology, College of Agriculture, Rajendranagar, Professor Jayashankar Telangana Agricultural University conducted during 2023-24

Methodology: A total of six fungicides viz., Captan 50 WP, Thiram 75% DS, Tebuconazole 5.36% FS, Carboxin 37.5% + Thiram 37.5% DS, Prochloraz 24.4% + Tebuconazole 12.1% EW and Thiophanate Methyl 450 g/l + Pyraclostrobin 50 g/l were evaluated at six different concentrations (100, 250, 500, 1000, 1500 and 2000 µg/mL) against three plant pathogens viz., *R. solani*, *M. phaseolina*, and *S. rolfsii* using the poisoned food technique. Mycelial growth inhibition was assessed after incubation for seven days.

Results: Among the tested fungicides, Carboxin 37.5% + Thiram 37.5% DS was the most effective demonstrating over maximum mycelial inhibition of all the three pathogens *R. solani*, *M. phaseolina* and *S. rolfsii* even at lower concentrations ranging from 86% (*M. phaseolina* at 100 µg/mL) to 100% (*R. solani* and *S. rolfsii* at 100 µg/mL). The next effective fungicide was Prochloraz 24.4% + Tebuconazole 12.1% EW at 100 and 250 µg/mL concentrations. Thiram 75% DS followed by Captan 50 WP were the least effective among the six fungicides. These findings suggest that Carboxin + Thiram and Prochloraz + Tebuconazole can be recommended as promising fungicidal treatments at 100-250 µg/mL for minimizing soil-borne fungal infections and ensuring healthy crop establishment.

Keywords: Soil-borne, fungicides, mycelial growth, poisoned food technique, *in vitro*

Introduction

The agricultural productivity across the globe is threatened by the incidence of various pests and diseases with estimated yield losses of 20-40% each year (Savary *et al.*, 2019) [10]. Plant diseases reduce yields by 21-30% in several important crops worldwide (El-Saadony *et al.*, 2022) [2]. Plant diseases caused by various seed and soil-borne pathogens determine the dynamics of plant populations in both natural and agricultural environments.

Soil-borne fungal pathogens are some of the most persistent and destructive constraints to global agricultural productivity. These pathogens survive for extended periods in soil as sclerotia, microsclerotia or mycelial fragments and initiate infection at the root, crown or collar regions of susceptible hosts, often leading to severe seedling mortality and substantial yield losses. Necrotrophic soil inhabitants such as *Rhizoctonia solani*, *Macrophomina phaseolina* and *Sclerotium rolfsii* are among the most damaging causal agents of sheath blight of rice, charcoal rot of maize, and stem rot of groundnut, respectively. Their wide host range, ability to survive under diverse agro-ecological conditions, and capacity to persist in soil for many years make them particularly challenging to manage (Panth *et al.*, 2020) [9].

Yield losses caused by soil-borne pathogens can reach 50-75% in several major crops, especially under environmental conditions that favour pathogen growth, survival, and infection processes (Savary *et al.*, 2019; El-Saadony *et al.*, 2022) [10, 2]. The resilience of these pathogens arises from their hardy survival structures—sclerotia and microsclerotia—which enable them to withstand unfavourable conditions and serve as primary inoculum across seasons. Once established, these pathogens cause root rot, collar rot, wilt, damping-off, and stem blight symptoms that severely impair plant growth and productivity. Host plant resistance is an ideal and eco-effective strategy for managing soil-borne diseases. However, frequent pathogenic variability and the necrotrophic nature of these fungi often limit the

durability of resistance (Monga *et al.*, 2018) [8]. Hence, integrated disease management approaches that combine host resistance with cultural, biological and chemical strategies are essential for achieving reliable disease suppression. Among these, chemical fungicides remain a key component due to their rapid and broad-spectrum action, ease of use, and effectiveness in reducing early-season disease establishment (Machado *et al.*, 2017) [6]. Seed treatment and soil application with fungicides are widely adopted to protect emerging seedlings from soil borne inoculum and to reduce primary infection foci. The efficacy of fungicides, however, varies with active ingredient, formulation, concentration and the specific pathogen targeted (Lamichhane *et al.*, 2020) [5]. Therefore, laboratory-based evaluations of fungicide performance under controlled conditions are critical before recommending field-level applications (Maitlo *et al.*, 2014) [7]. In this context, the present study was undertaken to evaluate the *in vitro* efficacy of commonly used fungicides against three major soil-borne fungal pathogens—*R. solani*, *M. phaseolina* and *S. rolfii*—to identify the most effective chemistries for their management.

Materials and Methods

The experiment was carried out in the Department of Plant Pathology, College of Agriculture, Rajendranagar, Professor Jayashankar Telangana Agricultural University (PJTU), Hyderabad, Telangana state, India during July-August, 2024. Pure cultures of three pathogens *Rhizoctonia solani*, *Macrophomina phaseolina* and *Sclerotium rolfii* were obtained from the Microbial Culture Collection Centre, Department of Plant Pathology, College of Agriculture, Rajendranagar, PJTU, Hyderabad, Telangana state, India (Table 1).

Evaluation of fungicides against *Fusarium* spp. under *in vitro* conditions

Six fungicides (Table 2), commonly used for seed treatment were selected from the pesticides recommended as per the Central Insecticides Board & Registration Committee, India (CIB & RC, 2024) [1] were tested for their efficacy against five pathogens (Table 1) at six concentrations *viz.*, 100 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL, 1500 µg/mL and 2000 µg/mL using the poisoned food technique (Nene and Thapliyal, 1993) under *in vitro* conditions.

Table 1: List of soil-and seed-borne pathogens used in the present study

S. No.	Name of the pathogen (disease caused)	Host crop	Place of collection	Latitude & Longitude
1.	<i>Rhizoctonia solani</i> (sheath blight)	Rice (<i>Oryza sativa</i>)	Kondapaka, Karimnagar 505502, Telangana	18.400, 79.441
2.	<i>Macrophomina phaseolina</i> (charcoal rot)	Maize (<i>Zea mays</i>)	Nagasala, Mahbubnagar 509301, Telangana	18.014, 79.603
3.	<i>Sclerotium rolfii</i> (stem rot)	Groundnut (<i>Arachis hypogaea</i>)	Rajendranagar, Hyderabad 500030, Telangana	17.331, 78.410

Table 2: List of the fungicides used in the present study

S. No.	Name of the fungicide	Trade name	Mode of action	Chemical group	Manufacturing company
1.	Captan 50 WP	Captaf	Non systemic Multi-site enzyme inhibition	Phthalimides	Rallis India Ltd.
2.	Thiram 75% WS	Seedcap	Non systemic Multi-site enzyme inhibition	Dithio-carbamate	Jaivik Crop Care Ltd.
3.	Tebuconazole 5.36% FS	Raxil	Systemic Inhibits ergosterol biosynthesis	Triazoles	Bayer Crop Science Ltd.
4.	Carboxin 37.5% + Thiram 37.5% DS	Vitavax power	Systemic & contact Succinate dehydrogenase inhibitor + multi-site inhibition	Anilide and dithiocarbamate	Dhanuka Agritech Ltd.
5.	Prochloraz 24.4% + Tebuconazole 12.1% EW	Zamir	Systemic Inhibits ergosterol biosynthesis	Imidazole + triazole	Adama Ind. Pvt. Ltd.
6.	Thiophanate methyl 450 g/l + Pyraclostrobin 50 g/l	Xelora	Systemic Inhibits β-tubulin assembly and mitochondrial respiration	Benzimidazole and Strobilurin	BASF Ind. Ltd.

Stock solution of 100000 ppm concentration was prepared using 10 ml of sterilized distilled water. Desired concentration of fungicide was obtained by diluting the stock solution using the following formula $C_1V_1 = C_2V_2$ Where, C_1 = concentration of the stock solution (mg/L), V_1 = volume of the stock solution to be added (mg/L), C_2 = desired concentration (mg/L) and V_2 = volume of Potato Dextrose Agar (PDA) in which fungicide is to be amended (ml).

The required quantity of each fungicide was added separately to the sterilized PDA medium, mixed thoroughly and poured in sterilized 90 mm diameter glass Petri plates and allowed to solidify. Three replications were maintained. A control plate was also maintained, where medium was not supplemented with any fungicides. Each plate was inoculated with 5 mm discs of 5 days old pathogen cultures

taken with the help of sterilized cork borer and incubated at $25 \pm 2^\circ\text{C}$ for 7 days. Mycelial growth of the pathogen was recorded daily in the control plate starting from the initiation of the pathogen growth in correspondence to treatment plates till the fungal growth was full in control. Based on the observations recorded, the per cent growth inhibition will be calculated by the formula as mentioned (Vincent, 1947)- $I = (C-T) \times 100/C$ where

I = Per cent inhibition of mycelial growth over control
 C = Mycelial growth of the pathogen in control (mm)
 T = Mycelial growth of the pathogen in treatment (mm)

Statistical Analysis

The data collected were analysed by analysis of variance (ANOVA) using the Statistical Package for Agricultural

Scientists (OPSTAT). Duncan Multiple Range Test (DMRT) was used to analyse the data.

Results and Discussion

The six fungicides showed significant variation in their efficacy against the five pathogens with distinct inhibition pattern noted for each pathogen.

Evaluation of fungicides on mycelial growth of *Rhizoctonia solani* under *in vitro* conditions

All the fungicides tested in this study significantly ($p \leq 0.05$) inhibited the mycelial growth of *R. solani* at varying concentrations when compared to the untreated control (Figure 1). Notably, carboxin 37.5% + thiram 37.5% DS and prochloraz 24.4% + tebuconazole 12.1% EW showed complete inhibition (100%) of mycelial growth at all concentrations ranging from 100 to 2000 $\mu\text{g/mL}$, indicating their broad-spectrum and potent fungitoxic action. Fungicides captan 50 WP and thiophanate methyl 450 g/L + pyraclostrobin 50 g/L FS also showed considerable suppression. Thiram 75% DS was the least effective with 0% inhibition at 100-500 $\mu\text{g/mL}$. However, once the fungicide concentration was at 1000 $\mu\text{g/mL}$ it was able to completely inhibit the mycelial growth. On the other hand, the effectiveness of Tebuconazole 5.36% FS was gradual across the concentrations with 81% inhibition at 1000 $\mu\text{g/mL}$ followed by 85% and 100% inhibition at higher concentrations. Recently, Vittal *et al.* (2025) [14] also demonstrated the broad-spectrum effectiveness of carboxin 37.5% + thiram 37.5% DS and prochloraz 24.4% + tebuconazole 12.1% EW against three *Fusarium species* (Vittal *et al.*, 2025) [14].

Evaluation of fungicides on mycelial growth of *Macrophomina phaseolina* under *in vitro* conditions.

In case of *M. phaseolina*, significant differences in the antifungal activity of the tested fungicides were observed with carboxin 37.5% + thiram 37.5% DS being the most effective fungicide achieving complete (100%) inhibition of mycelial growth at concentrations ranging from 250 to 2000 $\mu\text{g/mL}$ (Figure 2). This was followed by thiophanate methyl 450 g/L + pyraclostrobin 50 g/L FS exhibiting comparable inhibition at concentrations above 500 $\mu\text{g/mL}$. Except for thiram 75% DS, all the fungicides were effective against *M. phaseolina* at 1000 $\mu\text{g/mL}$ and above concentrations. The mycelia showed 0% inhibition at 100-500 $\mu\text{g/mL}$ concentrations and 11% inhibition at 1000 $\mu\text{g/mL}$ concentrations indicating that thiram was ineffective against

M. phaseolina. This clearly indicated the superior efficacy of thiram when used in combination with carboxin and also suggested the use of integrated management practices against soil-borne pathogens such as *M. phaseolina* (Vittal *et al.*, 2025) [14]. Recent studies by Khamari and Patra (2018) [4] and Karibasappa *et al.* (2020) [3] also confirmed the efficacy of the combination fungicide carboxin + thiram against *M. phaseolina* under *in vitro* conditions

Evaluation of fungicides on mycelial growth of *Sclerotium rolsii* under *in vitro* conditions

All the tested fungicides significantly ($p \leq 0.05$) suppressed the mycelial growth of *S. rolsii* across the concentration gradient when compared to the control (Figure 3). Among them, tebuconazole 5.36% FS, carboxin 37.5% + thiram 37.5% DS, prochloraz 24.4% + tebuconazole 12.1% EW and thiophanate methyl 450 g/L + pyraclostrobin 50 g/L FS completely inhibited mycelial growth (100%) at concentrations ranging from 250-2000 $\mu\text{g/mL}$. Interestingly, tebuconazole performed better against *S. rolsii* compared to *R. solani* and *M. phaseolina* even at lower concentrations of 100-500 $\mu\text{g/mL}$. This might be because *S. rolsii* grows fast with soft, actively expanding hyphae, that depend on continuous membrane (sterol) synthesis. Tebuconazole, being a DMI inhibits ergosterol synthesis, might be blocking this thus quickly slowing the fungal growth. In contrast, *M. phaseolina* and *R. solani* produce thicker and denser mycelia and early survival structures compared to *S. rolsii*, reducing their sensitivity to tebuconazole in PDA. Thiram 75% DS followed by Captan 50 WP however showed comparatively lower inhibition, indicating variability in fungitoxicity among the formulations. These results align with Shirsole *et al.* (2019) [11], who reported that Carboxin + Thiram completely inhibited *S. rolsii* under *in vitro* conditions. Similarly, Vamshi *et al.* (2019) [12] observed 100% inhibition of *S. rolsii* growth with Thiram and Tebuconazole, supporting the current findings.

The six fungicides showed clear differences in their sensitivity patterns against the three pathogens, which might be attributing to their contrasting biology and different fungitoxic potential of the fungicide molecules. Among them, carboxin 37.5% + thiram 37.5% DS and prochloraz 24.4% + tebuconazole 12.1% EW consistently produced complete inhibition across a wide concentration range, reflecting their strong systemic action and multi-site interference which agrees with earlier reports on other necrotrophs (Khamari and Patra, 2018; Vittal *et al.*, 2025) [18, 14].

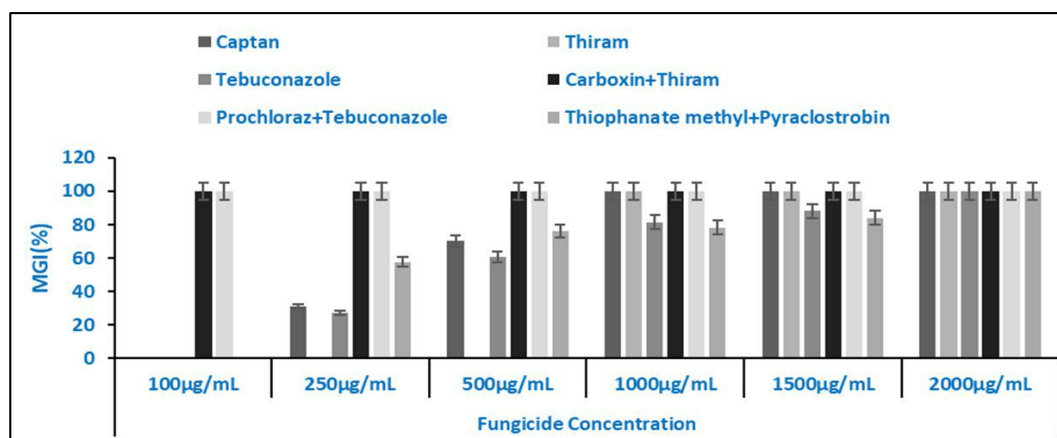


Fig 1: Efficacy of fungicides on per cent mycelial growth inhibition of *Rhizoctonia solani*

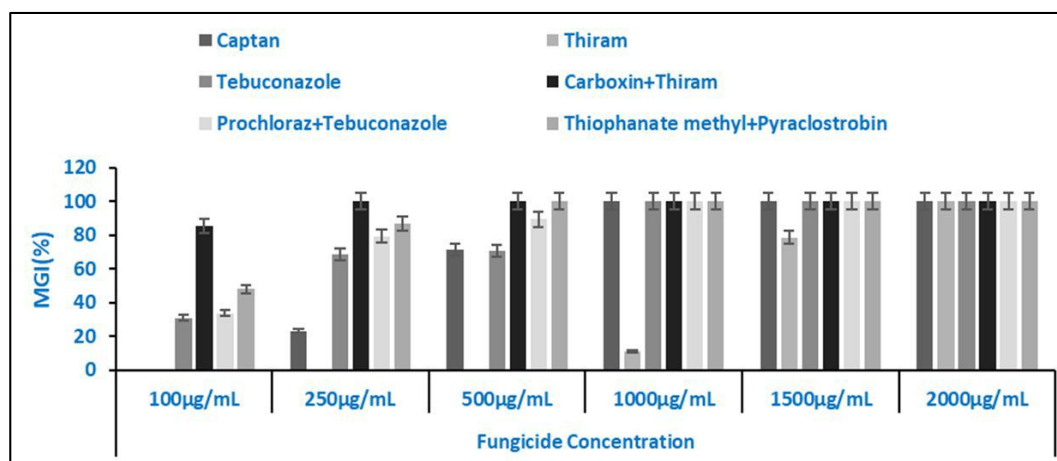


Fig 2: Efficacy of fungicides on per cent mycelial growth inhibition of *Macrophomina phaseolina*

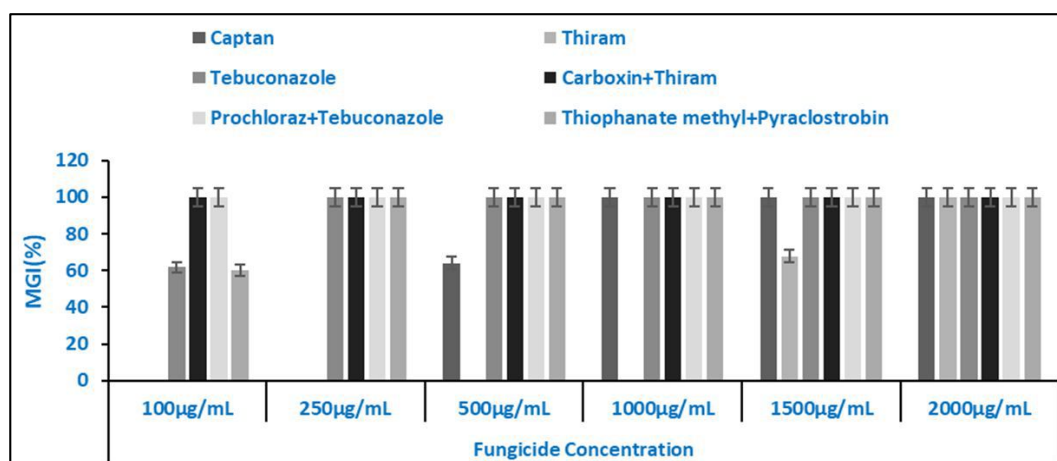


Fig 3: Efficacy of fungicides on per cent mycelial growth inhibition of *Sclerotium rolfsii*

Conclusion

The study revealed that fungicides varied significantly in their efficacy against major soil-borne pathogens under *in vitro* conditions. Among the tested fungicides, Carboxin 37.5% + Thiram 37.5% DS and Prochloraz 24.4% + Tebuconazole 12.1% EW showed complete inhibition of mycelial growth across all concentrations, indicating their superior and broad-spectrum antifungal activity. These formulations can be effectively recommended to manage soil-borne fungal pathogens, ensuring healthy crop establishment and improved productivity.

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