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# COMPARATIVE *IN VITRO* EFFICACY OF FUNGICIDES AND MICROBIAL ANTAGONISTS AGAINST *SCLEROTIUM ROLFSII* ASSOCIATED WITH FRUIT ROT OF SPONGE GOURD

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**ABSTRACT**

Sponge gourd (*Luffa aegyptiaca*) is an economically important cucurbit cultivated extensively in India for its edible fruits and fibrous mesocarp. Fruit rot incited by *Sclerotium rolfsii*, a destructive necrotrophic soil-borne pathogen, has recently emerged as a serious constraint to sponge gourd production. The disease is characterized by water-soaked lesions, progressive fruit decay, wilting, profuse white mycelial growth, and sclerotial formation. The present investigation was undertaken to evaluate the *in vitro* efficacy of selected fungicides and microbial antagonists against *S. rolfsii* isolated from diseased sponge gourd fruits collected from Uttar Pradesh, India, during 2024–25. Seven fungicides, namely Hexaconazole (5% SC), Propiconazole (25% EC), Propineb (70% WP), Tebuconazole + Trifloxystrobin (75 WG), Azoxystrobin (23% SC), Mancozeb (75% WP), and Copper oxychloride (50% WP), were tested at 50, 100, 500, and 1000 ppm using the poisoned food technique. The antagonistic potential of five isolates of *Trichoderma harzianum* (TH1–TH5), one isolate of *T. viride* (TV1) along with bacterial antagonists namely *Bacillus safensis*, *B. subtilis*, *Pseudomonas fluorescens* and a commercial *P. fluorescens* formulation was assessed through dual culture assays. The results revealed that Hexaconazole, Propiconazole, Propineb and Tebuconazole + Trifloxystrobin completely inhibited the mycelial growth of *S. rolfsii* at 500 ppm and above. Azoxystrobin exhibited moderate inhibitory activity (75–85%), whereas Mancozeb and Copper oxychloride were comparatively less effective, with inhibition ranging from 6 to 25%. Among the bacterial antagonists, *B. safensis* was the most effective, recording 76.8% mycelial growth inhibition, followed by *B. subtilis* (49.6%), while *Pseudomonas* strains exhibited relatively lower inhibition (28–35%). Among the fungal antagonists, *T. harzianum* isolate TH3 showed the highest antagonistic potential (85.5%), whereas the remaining *T. harzianum* isolates exhibited moderate inhibition (56–62%) and *T. viride* (TV1) was the least effective (50%). Overall, the study identifies promising chemical and biological options for the management of *S. rolfsii* and provides a scientific basis for developing integrated disease management strategies for fruit rot of sponge gourd.

***Key words :*** Sponge gourd, *Sclerotium rolfsii*, Bio agents and Fungicides.

## Introduction

Sponge gourd (*Luffa cylindrica* syn. *L. aegyptiaca*) belongs to the Cucurbitaceae family, and is a cultivated species of the genus Luffa is a fairly large vine bearing large, 8–10 cm wide, bright yellow flowers. The cylindrical, green fruits are harvested when young, 20 cm long, and usually boiled, but can achieve a length of 40 cm or so at maturation. They are also called smooth loofah or dishcloth gourd. The endocarp dries out as the fruit matures, forming a sponge that has a wide variety of uses. This fibrous net is the dried remains of the highly anastomosing vasculature of the fruit mesocarp. The sponge gourd is not a tropical plant and requires good care for accurate growth and harvest. Mainly it is grown in Indian states likes- Punjab, Bihar, U.P., Delhi, Gujarat, Haryana, Rajasthan and Jharkhand. Newly emerging fungal and fungi-like diseases are increasingly being reported in Sponge gourd including a wide range of hosts. New fungal diseases associated with sponge gourd are becoming a serious threat to its production. The pathogen *S. rolfsii* causes fruit rot and stem rot in Sponge gourd and produces symptoms like water-soaked and rotted fruit, yellowing and wilting of leaves, white mycelial mats spreading over lesions, sclerotia forming on fruit, leaves and near soil line. First report of sclerotium rot on Sponge gourd caused by *S. rolfsii* was in Korea (Kwon *et al*., 2012). *S. rolfsii* is a necrotrophic soil borne plant pathogen, killing plant tissues in advance of colonization by production of oxalic acid and cell wall degrading enzymes. It survives as sclerotia in soil and as mycelium in crop debris. Sclerotia are known to survive several years in the absence of a host. The extremely broad host range of *S. rolfsii* also contributes to long-term survival between host crops. *S. rolfsii* thrives in highly aerobic environments and thus survives best near the soil surface. Environmental conditions that are favourable for the fungus and disease development are high temperatures (27 to 35°C), humid conditions, and acidic soil. Germination of sclerotia occurs at pH 3 to 7 and is inhibited at pH levels higher than 7. Sclerotia are disseminated through the movement of infested soil, infected transplants and contaminated tools and machinery. Fruit rot in Sponge gourd is newly emerged disease. So, the information regarding the management aspect and host range is not much available. Keeping the view on these aspects, the present study is on management of *Sclerotium rolfsii* causing fruit rot in Sponge gourd through tested fungicides and native antagonist under laboratory conditions.

## Materials and Methods

The experiments were carried out at Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India (26°47′ N, 81°28′ E) in the year 2024–2025.

### Evaluation of different fungicides against fruit rot of Sponge gourd

Efficacy of seven fungicides *viz*., Hexaconazole 5% S.C. (Contaf plus), Azoxystrobin 23% S.C. (Mirador), Tebuconazole 50% + Trifloxystrobin 25% W.G. (Nativo), Propineb 70% W.P. (Antracol), Propiconazole 25% E.C. (Tilt), Mancozeb 75% W.P. (Indofil M-45) and Copper oxychloride 50% W.P. (Blitox) were tested against the mycelial growth of *S. rolfsii* at four different concentrations *i.e.*; 50 ppm, 100 ppm, 500 ppm and 1000 ppm. Poisoned food technique was employed to evaluate the efficacy of these chemicals. To get the final test concentration, the necessary amount was pipetted out and added to the necessary amount of molten PDA medium in conical flasks. Each Petri plate that had been previously sterilized was poured 20 ml of fungicide-amended media. 5 mm diameter mycelial disc using a sterile cork borer under aseptic condition was taken from a 15-day-old *S. rolfsii* culture and put into the center of each poisoned plate. Control plates were maintained by placing fungal discs without containing fungicides and served as untreated (non poisoned) medium. Three replications of each treatment were maintained with applying the CRD design of the experiment. Each petri plates were placed in BOD incubator at 27 ± 1°C for incubation. Observations on radial mycelial growth were recorded in all the treatment plates, after complete growth noticed in control plates and per cent inhibition was calculated by applying the formula given by Vincent (1947).

$$I = C\text{-}T/C \times 100$$

Where, I = Per cent inhibition of mycelial growth

C = Fungal growth in control (mm)

T = Fungal growth in treatment (mm)

### Evaluation of different fungal and bacterial antagonists against fruit rot in Sponge gourd

**Fungal antagonists :** Dual culture technique was applied to study the antagonistic effect of fungal bioagents. Efficacy of five isolates of *Trichodernma harzianum* (TH1, TH2, TH3, TH4, TH5) and one isolate of *Trichoderma viridae* (TV1) were tested against the mycelial growth of *S. rolfsii*. Sterilized Petri dishes were poured with 20 ml of chilled and sterilized PDA in order to assess antagonists *in vitro*. Fungal antagonists were evaluated by inoculating the pathogen on one side of the Petri plates and the fungal antagonist on the other side of the same plate, leaving a 3–4 cm space between them. For this, freshly cultivated cultures were used. After the necessary incubation time, or four days, or when the growth in the control plate reached 90 mm in diameter, the pathogen's radial growth was assessed. Per cent inhibition was calculated by applying the formula given by Vincent (1947) as mentioned above.

**Bacterial antagonists :** Dual culture technique was conducted to study the antagonistic effect of bacterial bioagents. Efficacy of two isolates of *Pseudomonas* spp. (*P. fluorescens* and *P. fluorescens* comm.), *Bacillus safensis* and *Bacillus subtilis* were tested against the mycelial growth of *S. rolfsii*. Sterilized Petri dishes were poured with 20 ml of chilled and sterilized PDA in order to assess antagonists *in vitro*. In the case of the bacterial antagonist, mycelial disc of the pathogen was injected at the centre, and the bacterial antagonist was streaked on both sides of fungus at equal distance. After the necessary incubation time, or four days, or when the growth in the control plate reached 90 mm in diameter, the pathogen's radial growth was assessed. Per cent inhibition was calculated by applying the formula given by Vincent (1947) as mentioned above.

## Results and Discussion

### Evaluation of fungicides

The *in vitro* evaluation of seven fungicides against *Sclerotium rolfsii* showed significant variation in radial mycelial growth and percent inhibition presented in Table 1. Among seven fungicides tested, the most effective fungicides were Hexaconazole 5% SC, Propiconazole 25% EC, Propineb 70% WP and Tebuconazole + Trifloxystrobin (Nativo), which showed 100% inhibition at 500 and 1000 ppm, with 0.00 mm radial growth. These treatments were found to be statistically at par with each other (CD for interaction = 1.10), indicating no significant difference in their efficacy at higher concentrations. Azoxystrobin 23% SC showed moderate inhibition (75.18%–85.36%) and was at par with lower concentrations (50–100 ppm) of Propiconazole and Nativo, but significantly less effective than their higher doses. Mancozeb 75% WP and Copper oxychloride 50% WP showing the least inhibition were significantly inferior to systemic fungicides like Hexaconazole and Propiconazole (Fig. 1). These findings are consistent with those of Sundar *et al*. (2006), who reported that systemic triazoles like hexaconazole and propiconazole are highly effective in suppressing *S. rolfsii* in vitro. The combination product Tebuconazole + Trifloxystrobin (Nativo) performed exceptionally well, aligning with the results of Rathod and Peshney (2011), who noted its synergistic effect in managing soil borne pathogens, including *S. rolfsii*. Similarly, Propineb, a dithiocarbamate fungicide, was effective at higher concentrations, corroborating the observations of Patel *et al*. (2014), who emphasized its potential against collar rot pathogens. Azoxystrobin 23% SC showed moderate inhibition, with increasing efficacy at higher doses, but did not achieve complete control. These results are similar to those of Kaur *et al*. (2018),

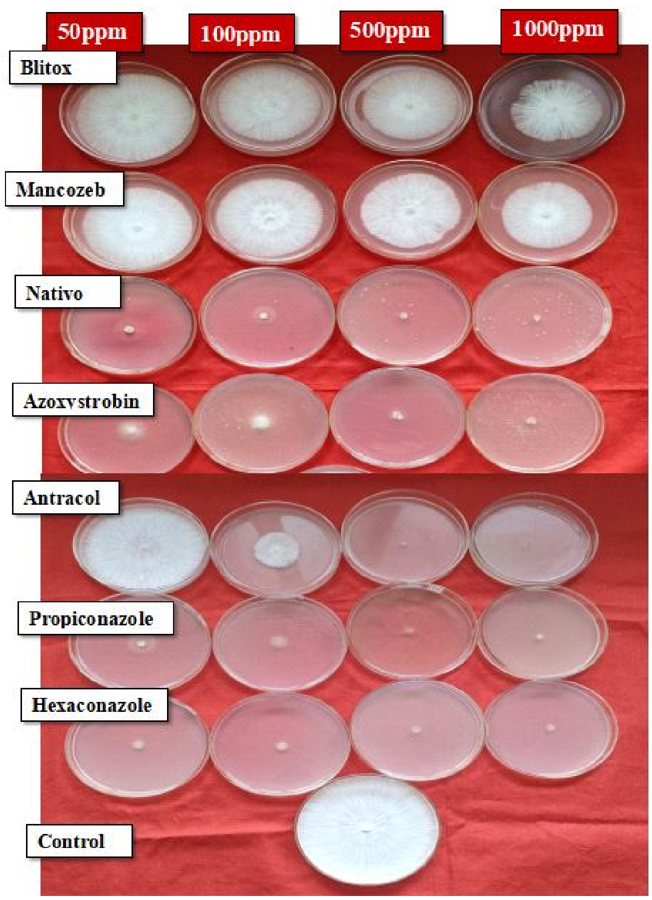


**Fig. 1 :** Evaluation of the efficacy of novel fungicides against *S. rolfsii* through poisoned food technique *in vitro*.

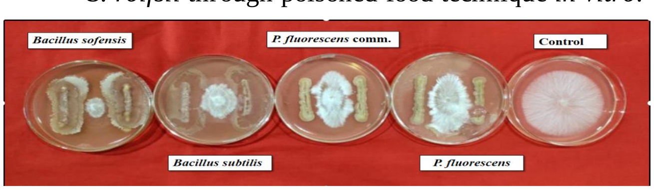


**Fig. 2 :** Radial growth of mycelium of *S. rolfsii* against bacterial antagonists.

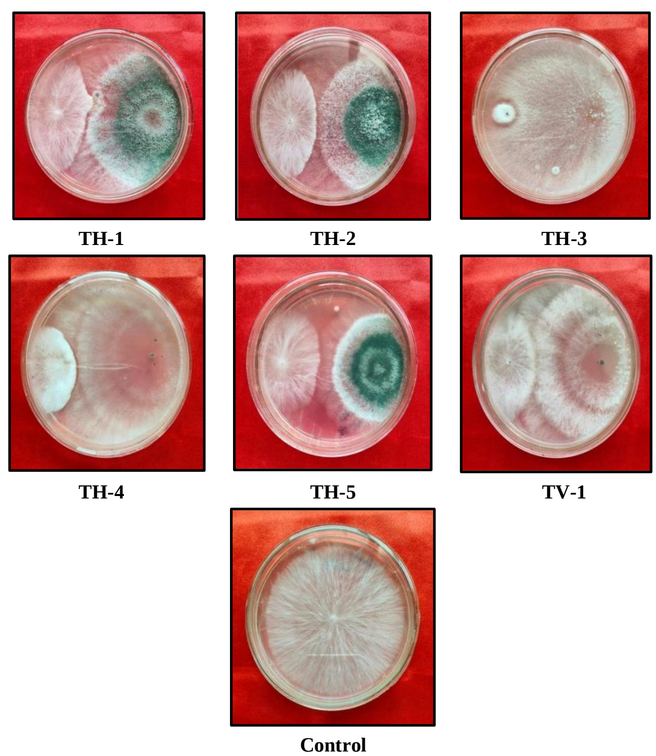


**Fig. 3 :** Radial growth of mycelium of *S. rolfsii* against isolates of fungal antagonists.

**Table 1 :** Evaluation of the efficacy of novel fungicides against *S. rolfsii* through poisoned food technique under *in vitro* conditions.

| S. no. | Common and trade name of fungicides | Doses (ppm) | Mycelium of *S. rolfsii* after 72 hrs. | Percent inhibition of mycelia growth of *S. rolsfii* over control |
|---|---|---|---|---|
| 1. | Copper oxychloride 50% W.P. (Blitox) | 50<br>100<br>500<br>1000 | 84.33<br>84.00<br>82.50<br>67.00 | 6.30 (2.87)<br>6.66 (2.75)<br>8.33 (3.05)<br>25.55 (5.15) |
| 2. | Mancozeb 75% W.P. (Indofil M-45) | 50<br>100<br>500<br>1000 | 84.17<br>82.50<br>75.83<br>23.00 | 6.47 (2.70)<br>8.33 (3.04)<br>15.74 (4.07)<br>74.44 (8.68) |
| 3. | Tebuconazole 50% + Trifloxystrobin 25% W.G. (Nativo) | 50<br>100<br>500<br>1000 | 23.83<br>19.67<br>0.00<br>0.00 | 73.52 (8.63)*<br>78.14 (8.89)<br>100.00 (10.05)<br>100.00 (10.05) |
| 4. | Azoxystrobin 23% S.C. (Mirador) | 50<br>100<br>500<br>1000 | 22.33<br>20.50<br>14.17<br>13.17 | 75.18 (8.73)<br>77.22 (8.84)<br>83.70 (9.23)<br>85.36 (9.29) |
| 5. | Propineb 70% W.P. (Antracol) | 50<br>100<br>500<br>1000 | 72.67<br>20.00<br>0.00<br>0.00 | 19.25 (4.50)<br>77.77 (8.87)<br>100.00 (10.05)<br>100.00 (10.05) |
| 6. | Propiconazole 25% E.C. (Tilt) | 50<br>100<br>500<br>1000 | 16.33<br>11.33<br>0.00<br>0.00 | 81.85 (9.10)<br>87.41 (9.40)<br>100.00 (10.05)<br>100.00 (10.05) |
| **7.** | Hexaconazole 5% S.C. (Contaf plus) | 50<br>100<br>500<br>1000 | 8.33<br>0.00<br>0.00<br>0.00 | 90.74 (9.58)<br>100.00 (10.05)<br>100.00 (10.05)<br>100.00 (10.05) |
| 8. | Control | | 90.00 | - |
| **CD for fungicide** | | | **1.56** | **0.18** |
| **SE(m) for fungicide** | | | **0.55** | **0.06** |
| **CD for concentration** | | | **1.10** | **0.13** |
| **SE(m) for concentration** | | | **0.39** | **0.05** |
| **CD for interaction** | | | **3.11** | **0.36** |
| **SE(m) for interaction** | | | **1.10** | **0.12** |

*Values in parentheses are square root transformed value.

who found that azoxystrobin alone provides partial suppression of *S. rolfsii* and may be more effective when used in combination with other fungicides. In contrast, Mancozeb 75% WP and Copper oxychloride 50% WP, both contact fungicides, showed limited efficacy even at the highest concentrations. This supports the findings of Gogoi and Ali (2003), who reported that contact fungicides have relatively lower systemic action and therefore limited effectiveness against deep-seated mycelial pathogens like *S. rolfsii*.

## Evaluation of bacterial antagonists

The evaluation of efficacy of bacterial antagonists against *S. rolfsii* data is presented in Table 2. Among the tested four bacterial antagonists (*Bacillus safensis*, *Bacillus subtilis*, *Pseudomonas fluorescens* comm. and *Pseudomonas fluorescens*), *Bacillus safensis* was

**Table 2 :** Evaluation of bacterial antagonists (*Bacillus* spp. and *Pseudomonas* spp.) against *S. rolfsii* through dual culture method.

| S. no. | Bacterial antagonists | Mycelial growth of *S. rolfsii* (mm) after 48 hrs. | Percent inhibition of mycelia growth of *S. rolsfii* over control |
|---|---|---|---|
| 1. | *Bacillus safensis* | 16.25 | 76.78 (61.17) * |
| 2. | *Bacillus subtilis* | 35.25 | 49.64 (44.78) |
| 3. | *Pseudomonas fluorescens* comm. | 45.25 | 35.35 (36.46) |
| 4. | *Pseudomonas fluorescens* | 50.25 | 28.21 (32.06) |
| 5. | Control | 70.00 | 0.00 |
| | C.D. | 1.73 | 1.74 |
| | C.V. | 2.68 | 2.56 |
| | SE(m) | 0.57 | 0.56 |

*Values in parentheses are arcsine transformed value.

**Table 3 :** Evaluation of fungal antagonists (*Trichoderma* spp.) isolates against *S. rolfsii*.

| S. no. | Bacterial antagonists | Mycelial growth of *S. rolfsii* (mm) after 48 hrs. | Percent inhibition of mycelia growth of *S. rolsfii* over control |
|---|---|---|---|
| 1. | *Trichoderma harzianum* (TH1) | 32.83 | 53.10 (46.76) * |
| 2. | *Trichoderma harzianum* (TH2) | 30.17 | 56.90 (48.95) |
| 3. | *Trichoderma harzianum* (TH3) | 10.17 | 85.47 (67.57) |
| 4. | *Trichoderma harzianum* (TH4) | 26.17 | 62.61 (52.29) |
| 5. | *Trichoderma harzianum* (TH5) | 27.17 | 61.18 (51.45) |
| 6. | *Trichoderma viridae* (TV1) | 35.00 | 50.00 (44.98) |
| 7. | Control | 70.00 | - |
| | **C.D.** | **1.52** | **1.39** |
| | **C.V.** | **2.60** | **1.48** |
| | **SE(m)** | **0.49** | **0.45** |

*Values in parentheses are arcsine value/ transformed value.

found to be the most effective antagonist inhibiting 76.78% growth of fungus followed by *Bacillus subtilis* (49.64%), *Pseudomonas fluorescens* comm. (35.35%) and *Pseudomonas fluorescens* was the least efficient bacterial antagonist inhibiting 28.21% growth of fungus (Fig. 2). This is in line with findings by El-Deeb *et al.* (2022), who reported strong antagonistic potential of *Bacillus subtilis* isolates from groundnut rhizosphere against *S. rolfsii*. Similarly, Yanti *et al.* (2021) demonstrated that *Bacillus* consortia could inhibit *S. rolfsii* growth by up to 85% in tomato. Muthukumar *et al.* (2019) also found that *Pseudomonas* spp. can be used as antagonist for *S. rolfsii*. Similar findings were reported by Sunkad *et al.* (2022).

### Evaluation of fungal antagonists

The evaluation of efficacy of fungal antagonists against *S. rolfsii*, data is presented in Table-3. Among six fungal antagonists (TH1, TH2, TH3, TH4, TH5 & TV1), the isolate TH3 recorded the lowest mycelial growth (10.17 mm) of *S. rolfsii* and the highest inhibition percentage (85.47%). TV1 was the least effective, showing only 50.00% inhibition. TH2 (56.90%), TH4 (62.61%), and TH5 (61.18%) showed moderate levels of inhibition (Fig. 3). Similar findings were reported by Benítez *et al.* (2004), Kubicek *et al.* (2001) and Harman (2000).

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### Conflict of interest

There is no conflict of interest in the manuscript.

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