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In-vitro evaluation of seed-dressing fungicides for the management of *Sclerotium rolfii* Sacc. and enhancement of seed quality in groundnut

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Abstract

This study evaluated the efficacy of new-generation seed-dressing fungicides against *Sclerotium rolfii* in groundnut, along with their influence on seed quality parameters. *in vitro* experiments were conducted at the Seed Pathology Laboratory, Department of Seed Science and Technology, Odisha University of Agriculture and Technology, Bhubaneswar, using the poisoned food technique. Amongst the fungicides evaluated, Penflufen 13.28% + Trifloxystrobin 13.2% FS (Ever Golxtend) at 1%, Carboxin 37.5% WS + Thiram 37.5% WS (Vitavax Power) at 2% and Trifloxystrobin 25% + Tebuconazole 50% WG (Nativo) at 3% completely inhibited mycelial growth of the pathogen, whereas Thiophanate methyl 45% + Pyraclostrobin 5% FS (Xelora) proved least effective. In terms of seedling development, seed treated with Vitavax Power recorded the highest germination percentage, seedling length and vigour index, followed closely by Ever Golxtend and Xelora. Nativo, despite its strong antifungal activity, did not have significant effect on the seed quality parameters. These findings suggest that Vitavax Power and Ever Golxtend offer the best combination of disease control and seed quality enhancement for groundnut seed treatment.

Keywords: Groundnut, stem rot, new generation fungicides and seed quality

Introduction

Groundnut (*Arachis hypogaea* L.) considered as a major tropical and subtropical oilseed crop known for food, feed and edible oil because of its kernels are rich in protein, oil, vitamins, minerals and other bio-active compounds (Krishnan *et al.*, 2025) [12]. It remains an economically important crop worldwide, with global production of about 54.2 million tonnes and India contributing nearly 10.1 million tonnes, establishing its importance in Indian agriculture (Jalla *et al.*, 2024 and Krishnan *et al.*, 2025) [9, 12]. However, the productivity of groundnut is being affected by numerous abiotic and biotic stresses, among which fungal diseases are especially considered more damaging (Deepika *et al.*, 2025) [4].

Among the various soil-borne diseases, groundnut stem rot or white mold caused by *Sclerotium rolfii* is found to be one of the most destructive diseases of groundnut in India and other hot and humid regions (Deepika *et al.*, 2025; Lavanya *et al.*, 2024) [4, 14]. The pathogen survives in soil for long periods through resilient sclerotia upon a broad host range and becomes difficult to manage under warm, humid conditions (Deepika *et al.*, 2025; Meena *et al.*, 2024) [4, 18]. Yield losses commonly range from 10-25%, but severe infections can cause losses of 40-55% or even up to 80% (Jalla *et al.*, 2024; Hotkar *et al.*, 2025) [7, 9]. Recent *in vitro* studies show strong activity of seed-treatment fungicides viz., carboxin + thiram and tebuconazole + trifloxystrobin against *S. rolfii* (Meena *et al.*, 2024; Surwade *et al.*, 2024) [18, 26]. Hence, the study was taken up to evaluate new-generation seed dressing fungicides against *S. rolfii* and their effects on seed quality parameters.

2. Material and Methods

The study was carried out during kharif 2024 in the Seed Pathology Laboratory, Department of Seed Science and Technology, OUAT, Bhubaneswar.

The causal organism of stem rot of groundnut, *Sclerotium rolfsii*, was isolated from infected seeds of the high yielding variety Dharani collected from farmers' fields in Khordha district, Odisha. Seed-borne fungi were detected by using the standard blotter paper method. The pathogen was purified by the hyphal tip culture method (Kedar *et al.*, 2025; Naikwade *et al.*, 2025; Yadav *et al.*, 2026) [10, 19, 29].

2.1 Evaluation of seed dressing fungicides under laboratory condition

The *S. rolfsii* pure culture was maintained on potato dextrose agar (PDA) media. The fungicides were evaluated following the poisoned food technique (Table 1). Each fungicide with the required dose was mixed with sterilized PDA before pouring the medium into Petri plates (Aravind *et al.*, 2024; Harshitha and Palakshappa, 2023; Satapathy and Sahoo, 2023) [2, 6, 23].

Table 1: Details of fungicide treatments evaluated against *Sclerotium rolfsii*

Treatment	Fungicide with formulation/concentration
T1	Penflufen 13.28% + Trifloxystrobin 13.2% FS
T2	Thiophanate methyl 45% + Pyraclostrobin 5%
T3	Trifloxystrobin 25% + Tebuconazole 50% WG
T4	Carboxin 37.5% WS + Thiram 37.5% WS
T5	Untreated control (PDA without fungicide)

After solidification of the medium, a 6 mm fresh culture with mycelial disc was cut from the growing edge and placed at the centre of each plate. The plates were then incubated in a BOD incubator for seven days. When the fungus in the control plates had nearly reached the edge of the plate, the colony growth was recorded (Aravind *et al.*, 2024; Leona *et al.*, 2020; Satapathy and Sahoo, 2023; Sigdel *et al.*, 2022) [2, 15, 23, 25].

Measurement of colony diameter was taken in two directions at right angles and the mean value was obtained. Inhibition (%) of mycelial growth over the control was calculated using the following formula:

$$\text{Growth inhibition (\%)} = \frac{C - T}{C} \times 100$$

Where, C is the colony growth (mm) in the untreated control and T is the colony growth (mm) in the fungicide treatment (Aravind *et al.*, 2024; Harshitha and Palakshappa, 2023) [2, 6].

2.2 Seed quality assessment

The assessment of the effect of fungicidal seed dressing on seed quality of groundnut was observed at 7 and 12 days after treatment. Seed health parameters were examined by using the standard blotter paper method, while germination and seedling growth were evaluated by the paper towel method (Gupta *et al.*, 2023; Naikwade *et al.*, 2025; Patil *et al.*, 2026) [5, 19, 20].

The experiment was conducted by laying out in a completely randomized design with four replications. Observations on different growth parameters viz., seed infection, germination and seedling were recorded regularly.

2.3 Growth and vigour measurements

Seeds treated with fungicides were evaluated for germination and seedling growth on the 12th day after sowing following the paper towel method. Each treatment

was replicated four times taking 100 seeds each. Germination percentage was calculated by selecting only the number of normal seedlings as follows:

$$\text{Seed germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds sown}} \times 100$$

Ten normal seedlings from each treatment and the untreated control were selected randomly, and their root and shoot lengths were measured. By adding the mean root length and mean shoot length, the mean seedling length was calculated and expressed in centimetres (Khan *et al.*, 2022; Kulik, 1977; Patil *et al.*, 2026; Pereira de Paiva *et al.*, 2019) [11, 13, 20, 21].

To determine the seedling dry weight the same 10 seedlings were used. After removing the remnants of seed coats, the seedlings were dried in a hot-air oven at 80 °C for 24 h, then removed and cooled to room temperature and weighed. Seedling dry weight was recorded and expressed in milligrams (mg) per seedling. The seedling lengths were averaged and mean seedling length observations were subsequently used and calculated the seed vigour indices.

2.4 Seed Vigour Indices (SVI) and statistical analysis

Seed vigour indices were calculated and assessed the effect of fungicidal seed treatments on seed germination and seedling growth. Seed Vigour Index-I (SVI-I) and Seed Vigour Index-II (SVI-II) were calculated following the method of Abdul-Baki and Anderson (1973) [1].

SVI-I = Germination (%) × Mean seedling length (cm) and

SVI-II = Germination (%) × Mean seedling dry weight (mg)

The vigour indices obtained were used to compare the overall physiological performance of seedlings under different fungicidal seed treatments with the normal seedling length under untreated control.

The recorded data were analyzed by computing the mean for each character in a Completely Randomized Design (CRD) and were subjected to analysis of variance (ANOVA) following the methods described by Rani *et al.* (2024) [22].

3. Result and Discussion

3.1 Effect of seed dressing fungicides on the growth of *Sclerotium rolfsii* under *in vitro* condition

Table 3 showed significant differences in the *in vitro* efficacy of the fungicides against *Sclerotium rolfsii*. Penflufen 13.28% + trifloxystrobin 13.2% FS (T1), trifloxystrobin 25% + tebuconazole 50% (T3) and carboxin 37.5% + thiram 37.5% WS (T4) completely inhibited mycelial growth and recorded 100% inhibition. A similar pattern has been reported in groundnut and soybean, where penflufen + trifloxystrobin, carboxin + thiram and trifloxystrobin + tebuconazole caused complete suppression of *S. rolfsii* under poisoned food assays (Vijaykumar *et al.*, 2023; Surwade *et al.*, 2024; Yadav *et al.*, 2025) [26,27,28].

In contrast, thiophanate methyl 45% + pyraclostrobin 5% (T2) was the least effective treatment, with 64.16% inhibition. This lower efficacy is consistent with reports that some thiophanate-methyl-based treatments provide substantially lower suppression than the best-performing combinations and that thiophanate methyl alone can show only partial inhibition depending on concentration and formulation (Butt *et al.*, 2021; Surwade *et al.*, 2024; Yadav *et al.*, 2025) [3, 26, 28].

The complete inhibition produced by trifloxystrobin + tebuconazole agrees with previous studies in groundnut, where this combination gave maximum inhibition *in vitro* and also performed well under field conditions (Meena and Meena, 2023; Surwade *et al.*, 2024) ^[17, 26]. Similar evidence from another stem rot pathosystem found that trifloxystrobin + tebuconazole had the strongest inhibitory effect, with very low EC50 values of 0.13-0.14 µg/mL, supporting the high intrinsic antifungal activity of this mixture (Zhao and Huang, 2023) ^[31].

The strong activity of carboxin + thiram also corroborates earlier reports in groundnut, where this mixture consistently inhibited the pathogen completely and was considered among the most effective fungicides (Surwade *et al.*, 2024; Yadav *et al.*, 2025) ^[26, 28]. Likewise, the penflufen + trifloxystrobin combination was reported to inhibit 100% mycelial growth of *S. rolf sii* across different concentrations, supporting the present finding that this seed-treatment combination is highly potent *in vitro* (Vijaykumar *et al.*, 2023) ^[27].

The superior performance of these combinations may be due to their multiple modes of action. The disruption of mitochondrial respiration by strobilurin components such as trifloxystrobin and pyraclostrobin occurs through the blockage of electron transfer at cytochrome b, thereby halting fungal energy production (Yadav *et al.*, 2025) ^[28]. Pyraclostrobin has also been shown to reduce sclerotial production, oxalate secretion and protein synthesis in *S. rolf sii*, which helps explain its preventive activity (You *et al.*, 2021) ^[30].

3.2 Effect of seed dressing fungicides on seed germination

Fungicide seed dressing significantly enhanced groundnut germination over the untreated control (56.75%). Carboxin 37.5% WS + thiram 37.5% WS (T4, 81.75%) and penflufen 13.28% + trifloxystrobin 13.2% FS (T1, 80.50%) were the best-performing fungicides. The present findings were aligned with Asha *et al.* (2011) ^[24], who reported significantly higher seedling emergence (80.53-93.68%) with carboxin + thiram-treated groundnut seed over the untreated control (75.97%), and corroborated the earlier results of Jadon *et al.* (2015) ^[8], who found carboxin-and tebuconazole-based seed dressings effective in improving groundnut stand establishment. The germination gain likely reflects suppression of seed-borne mycoflora causing pre-emergence rot, similar to Manikchandra *et al.* (2026) ^[16], who recorded a rise in germination from 64.00% in the control to 90.67% following fungicidal seed dressing in Niger.

3.3 Effect of seed dressing fungicides on seedling length and seedling dry weight

Fungicide-treated groundnut seed produced longer seedlings than untreated controls, with carboxin 37.5% WS + thiram 37.5% WS (24.90 cm) recording the maximum length, followed by penflufen + trifloxystrobin (22.28 cm), while trifloxystrobin + tebuconazole gave the shortest seedlings (20.06 cm). This trend agrees with Asha *et al.* (2011) ^[24], who reported that carboxin + thiram (Vitavax Power) seed treatment in groundnut significantly increased root and shoot length and plant vigour over the check, attributing this to reduced pre-and post-emergence rot and improved plant establishment.

3.4 Effect of seed dressing fungicides on seedling vigour indices

Seedling vigour indices (SVI-I and SVI-II) differed significantly among fungicide treatments, with carboxin 37.5% WS + thiram 37.5% WS recording the highest SVI-I (1793.93) and SVI-II (296.56), while trifloxystrobin 25% + tebuconazole 50% WG gave the lowest values. Since vigour indices are derived from germination percentage combined with seedling length and dry weight, the superiority of carboxin + thiram reflects its combined advantage in both germination and seedling growth traits observed earlier. This finding is aligned with Asha *et al.* (2011) ^[24], who reported that carboxin + thiram seed treatment in groundnut improved plant vigour and establishment over untreated seed, attributing it to better protection of the germinating embryo from soil-and seed-borne pathogens. Similarly, Manikchandra *et al.* (2026) ^[16] observed that effective fungicidal seed dressing markedly raised the seedling vigour index over the untreated control in the rolled paper towel method.

3.5 Effect of fungicides on seedling length and seedling dry weight

Systemic fungicide seed treatments enhanced seedling growth over untreated seed, though the magnitude varied with the molecule. Carboxin 37.5% WS + thiram 37.5% WS produced the longest seedlings (24.90 cm), followed by penflufen + trifloxystrobin (22.28 cm) and thiophanate methyl + pyraclostrobin (20.73 cm), while trifloxystrobin + tebuconazole gave the shortest seedlings (20.06 cm). The results are in line with Asha *et al.* (2011) ^[24], who reported that carboxin + thiram seed dressing in groundnut significantly increased root and shoot length and overall seedling vigour compared with the check, owing to reduced pre-and post-emergence rot.

Table 2: Effect of seed dressing fungicides on mycelial growth (colony diameter) and growth inhibition of *Sclerotium rolf sii* under *in vitro* conditions

Treatment Fungicide (Trade name)	Concentration (g or mL L ⁻¹ of medium)	Colony diameter (mm) after 14 days*	Growth inhibition (%)
T1-Penflufen 13.28% + Trifloxystrobin 13.2% FS (EverGol Xtend)	1.0	0	100
T2-Thiophanate-methyl 45% + Pyraclostrobin 5% FS (Xelora)	1.0	32.25 (34.09)	64.17
T3-Trifloxystrobin 25% + Tebuconazole 50% WG (Nativo)	0.3	0	100
T4-Carboxin 37.5% WS + Thiram 37.5% WS (Vitavax Power)	2.0	0	100
T5-Control	—	90.00 (71.57)	0
SEm (±)		0.28	—
CD (P = 0.05)		0.85	—

*Values in parentheses are transformed arcsine value

Table 3: Effect of systemic fungicides on seedling length and seedling dry weight

Treatment Fungicide (Trade name)	Concentration (g or mL L ⁻¹ of medium)	Mean seedling length (cm)	Seedling dry weight (mg)
T ₁ -Penflufen 13.28% + Trifloxystrobin 13.2% FS (EverGol Xtend)	1.0	22.28	3.68
T ₂ -Thiophanate-methyl 45% + Pyraclostrobin 5% FS (Xelora)	1.0	20.73	3.10
T ₃ -Trifloxystrobin 25% + Tebuconazole 50% WG (Nativo)	0.3	20.06	2.91
T ₄ -Carboxin 37.5% WS + Thiram 37.5% WS (Vitavax Power)	2.0	24.90	4.00
T ₅ -Control	—	18.38	2.69
SEm (±)		0.63	0.35
CD (P = 0.05)		1.90	1.05



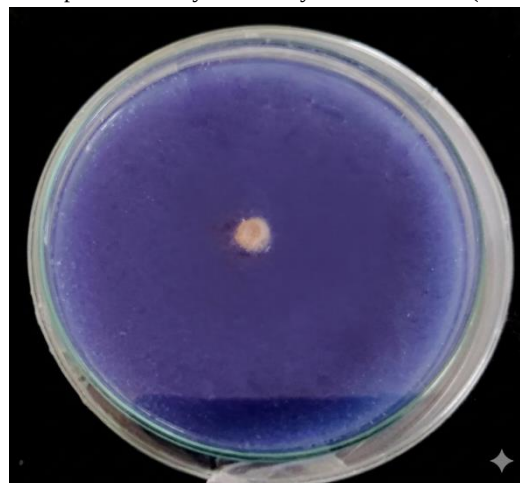
T₁-Penflufen 13.28% + Trifloxystrobin 13.2% FS (Ever Golxtend)



T₂-Thiophanate methyl 45% + Pyraclostrobin 5% (Xelora)



T₃-Trifloxystrobin 25% + Tebuconazole 50% (Nativo)



T₄-Carboxin 37.5% WS + Thiram 37.5% WS (Vitavax power)



T₅-Control

Fig 1: Effect of fungicides on the mycelial growth of *S. rolfsii*

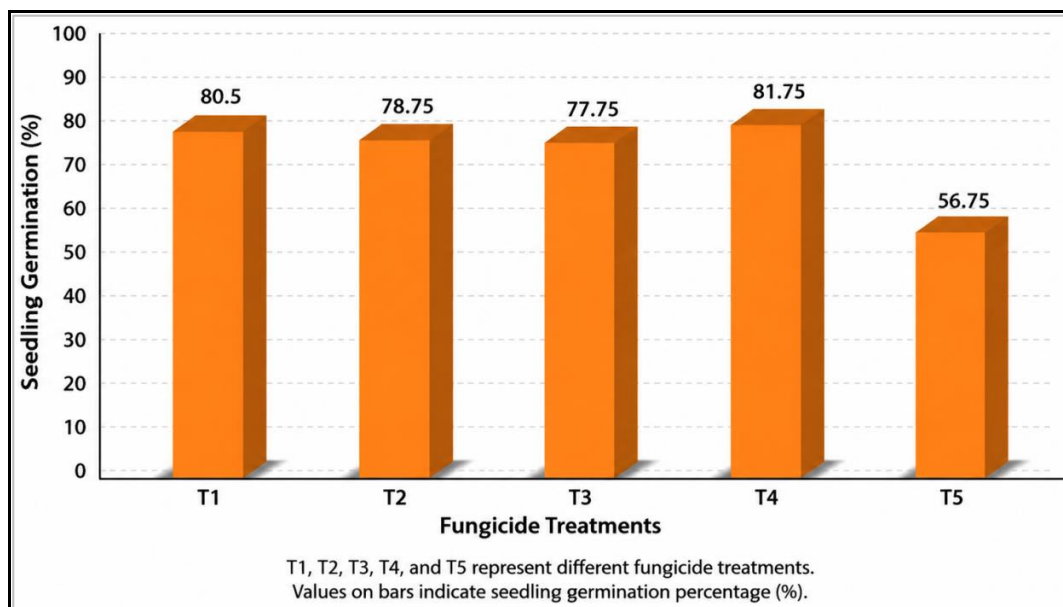


Fig 2: Effect of fungicides on seedling germination

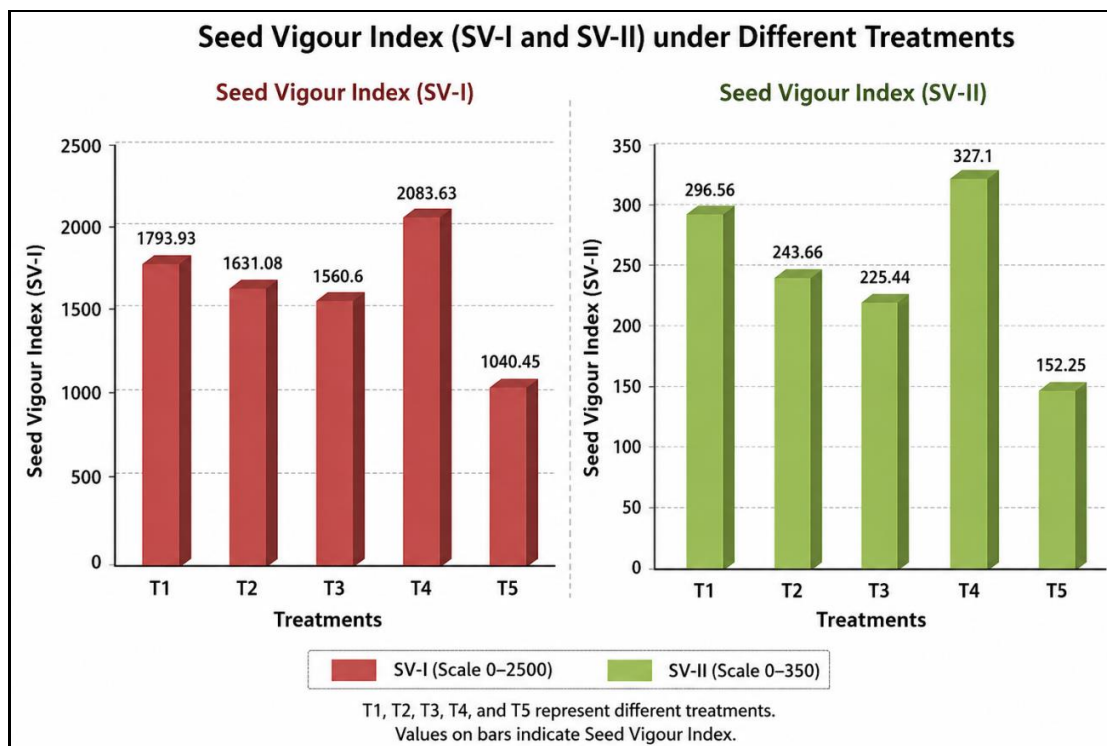


Fig 3: Effect of fungicides on Seedling Vigour-I and II

4. Conclusion

The study concluded that systemic fungicide molecules differed markedly in their ability to suppress stem rot fungus and improve groundnut seed quality. Penflufen + trifloxystrobin, carboxin + thiram and trifloxystrobin + tebuconazole completely inhibited fungal growth under *in vitro* conditions. Among the seed quality treatments, carboxin + thiram performed best, recording the highest germination, seedling length and vigour indices. Penflufen + trifloxystrobin and thiophanate methyl + pyraclostrobin also improved seed performance over other treatments. In contrast, trifloxystrobin + tebuconazole showed comparatively lower values for seed quality parameters despite strong antifungal activity. Overall, carboxin + thiram emerged as the most promising fungicidal seed treatment for managing stem rot and enhancing seed quality.

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