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RESEARCH ARTICLE

Comparative *in vitro* efficacy of fungicides and homoeopathic preparations against *Sclerotinia sclerotiorum* (Lib.) de Bary in ajwain

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Abstract

Ajwain (*Trachyspermum ammi* L.) Sprague is a high-value seed spice crop in India with considerable medicinal and culinary importance, yet its production is severely limited by stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, resulting in yield losses exceeding 50–80 %, with major economic implications for farmers and the spice export market. This study tested the hypothesis that selected homoeopathic formulations can reproducibly suppress fungal growth, providing environmentally safe and economically viable alternatives to chemical fungicides. *In vitro* evaluation revealed complete inhibition of *S. sclerotiorum* by propiconazole 11.9 % + azoxystrobin 7.1 % (Apropo), tebuconazole 18.3 % + azoxystrobin 11 % (Custodia) and difenoconazole 11.4 % + azoxystrobin 18.2 % (Amistar Top) at 150 ppm, while hexaconazole 5 % emulsifiable concentrate (EC), propiconazole 25 % EC and azoxystrobin 23 % suspension concentrate (SC) showed substantial inhibition. Among homoeopathic treatments, *Calcarea carbonica* and *Thuja occidentalis* achieved over 70 % inhibition. By integrating experimental results with published literature, patents and prior studies, this work provides a robust, interdisciplinary framework connecting plant pathology, homoeopathy and sustainable agriculture. The findings reveal a breakthrough: ultra-diluted biological preparations can complement fungicides, reduce chemical dependency, mitigate resistance development and support low-input, organic and export-oriented spice production systems. Economically, adoption of these strategies could protect yield worth millions of USD annually, while minimising environmental and regulatory risks. Future research should focus on field validation, application optimisation, biochar integration and techno-economic modelling, ensuring global applicability and maximising agronomic, ecological and financial benefits.

Keywords: ajwain; fungicides; homoeopathic drugs; stem rot

Introduction

Seed spices constitute a high-value segment of horticultural crops, contributing significantly to agricultural income, export revenue and agro-industrial value chains in semi-arid regions. India is the world's largest producer and exporter of seed spices, supplying both domestic and international markets. Ajwain (*Trachyspermum ammi* L.) Sprague; Apiacea is an annual seed spice cultivated primarily for its aromatic fruits and essential oil (1). Production is indicated in thousand tonnes (10^3 t), productivity in kilograms per hectare (kg ha^{-1}) and cultivation area in thousand hectares (10^3 ha). With an average yield of 1041 kg ha^{-1} and a production of 39.76×10^3 t, ajwain occupies roughly 38.18 $\times$ 10^3 ha in India, demonstrating its agronomic and economic significance (2). Ajwain seeds are rich in essential oil, dominated by thymol (35–60 %), a monoterpenoid phenolic compound responsible for antimicrobial, anti-inflammatory, analgesic, anxiolytic and antispasmodic activities (3, 4). Other components include γ-terpinene (~23.9 %) and p-cymene (~22.9 %), which improve both economic value and therapeutic efficacy (5). Residue-free production is a crucial prerequisite since it is immediately used as a food ingredient and pharmaceutical raw material, thereby connecting crop protection techniques to food safety, regulatory compliance and market acceptance. Fungal diseases including leaf spot, wilt, root rot, powdery mildew and stem rot severely limit ajwain productivity despite its capacity to adapt to arid and saline soils (6). One of the most damaging among them is stem rot brought on by *Sclerotinia sclerotiorum* (Lib.) de

Bary. *Sclerotinia sclerotiorum* is a necrotrophic ascomycete that may infect more than 400 different plant types causing significant yield losses in Assam, Rajasthan, Bihar, Uttar Pradesh, Uttarakhand and Haryana (7). The pathogen enters host tissues by wounds or natural holes, multiplies in cool, humid environments and causes host cell death by secreting enzymes that break down cell walls, such as pectinases, cellulases, hemicellulases and proteases (8). Water-soaked lesions, tissue maceration, wilting and the development of white, cottony mycelium with black sclerotia which act as long-term survival structures in soil are typical signs. In favourable conditions, yield losses from *S. sclerotiorum* in seed spices typically range from 20–35 %. In humid or temperate conditions, these losses might surpass 50 % and in extreme cases, they may result in complete crop failure (9–12). Crop rotation is less effective due to the polyphagous nature of the pathogen and long-term disease control is made more complicated by its persistence in the soil. Chemical fungicides are still the major method of management, but their prolonged usage is linked to pathogen resistance, environmental contamination, residue build up in edible plant portions and rising production costs. Given its direct intake and medicinal uses, these limitations are especially important for ajwain. Crucially, acceptance at the farm and industrial sectors cannot be guaranteed by environmental sustainability alone. Technologies for plant protection must also exhibit economic sustainability, which is characterised by lower investment risk, cost-effectiveness, yield stability and regulatory compatibility. Regardless of their ecological advantages, agricultural inventions with unpredictable profitability face limited commercialisation, as indicated by analyses of private investment behaviour. Therefore, an integrated environmental-economic approach must be used to assess disease management measures for seed spices. Homoeopathic remedies are a viable substitute for traditional fungicides in this situation. These inputs have very low application rates, little effect on the environment and very little risk of residue. However, because to a lack of comparable evidence on biological efficacy, their use in crop protection is still restricted. The current study postulates that specific homoeopathic formulations can provide improved environmental safety and economic viability while inhibiting *S. sclerotiorum* growth *in vitro* at levels comparable to commercial fungicides. Given the growing regulatory pressure on chemical inputs, rising production costs and the need for sustainable and lucrative disease management measures in seed spice farming, it is important and relevant to address this issue.

Materials and Methods

Molecular identification

The pure culture (*Sclerotinia* stem rot of Ajwain 2) was sent to Barcode Bioscience (Dr. Shivrama Karanth Nagar, Bangalore, India) for molecular identification and verification (13), which is shown in the Fig. 1.

Pathogenicity test

Pathogenicity was tested using the cotton mycelium method. Slightly moistened cotton containing a 5 mm mycelial disc from a fresh culture was placed at the stem node and wrapped with parafilm. Based on morphological features, the fungus was identified as *S. sclerotiorum* (14, 15).

In vitro efficacy of fungicides against *Sclerotinia sclerotiorum*

Seven fungicides of concentrations 50, 100, 150 ppm were used to assess their potential for inhibiting the growth of *S. sclerotiorum in vitro*. The evaluation was conducted using the poisoned food technique to determine the effectiveness of these drugs in controlling the pathogen (16). The fungicides used are hexaconazole 5 % EC (Contaf), azoxystrobin 23 % SC (Amistar), propiconazole 25 % EC (Tilt), propiconazole 11.9 % + azoxystrobin 7.1 % w/w SE (Suspo Emulsion) (Apropo), tebuconazole 18.30 % + azoxystrobin 11 % w/w SC (Custodia), difenoconazole 11.4 % + azoxystrobin 18.20 % w/w SC (Amistar Top) with carbendazim 50 % Wettable Powder (WP) (Bavistin) as positive check. All the fungicides were bought from Crop Care Pesticides India Pvt Ltd, Mohali.

For preparation of stock solution of 10000 ppm 0.5 mL/0.5 g of the fungicides with liquid/powder formulations were measured and dissolved in 50 mL of water. For preparation of working solutions to be used for poisoned food technique the measurements for 50, 100 and 150 ppm solutions are as calculated with formula $C_1V_1 = C_2V_2$. The required volumes of stock solution for each fungicide viz., 0.3, 0.6 and 0.9 mL to achieve concentrations of 50, 100 and 150 ppm, respectively were aseptically added to 60 mL of sterilised and melted potato dextrose agar (PDA) and thoroughly mixed. 20 mL of the prepared medium was then poured into each sterilised Petri dish. After solidification, a 5 mm disc of *S. sclerotiorum* was inoculated at the center of each plate, which was incubated at 19 ± 2 °C. The experiment was carried out using a completely randomised design (CRD) with three replications. The percentage of inhibition was determined by assessing the fungal mycelial growth on the

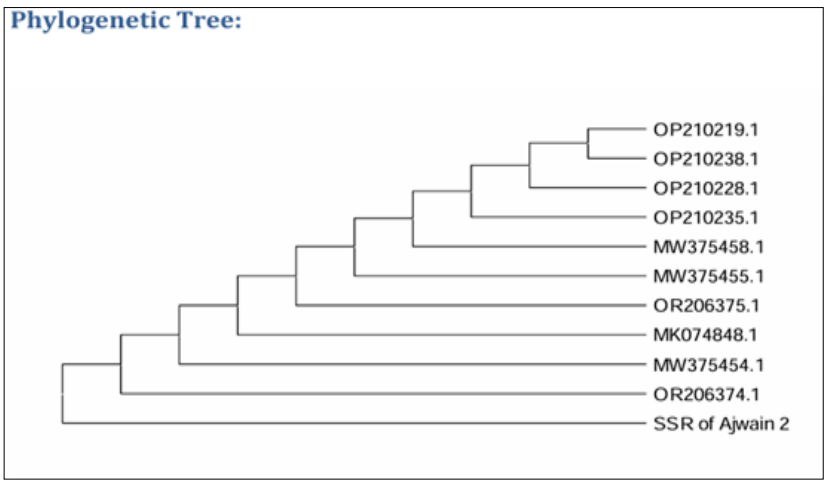


Fig. 1. Phylogenetic relationship between isolate SSR of ajwain 2 (NCBI PX309100) and other isolate.

treated plates compared to the control, using the following formula (17):

$$\text{Inhibition (\%)} = (C\text{-}T)/C \times 100 \qquad \text{(Eqn. 1)}$$

Where, C = diameter of fungal growth in control (mm); T = diameter of fungal growth in treated plate (mm)

In vitro efficacy of homoeopathic drugs against *Sclerotinia sclerotiorum*

Seven homoeopathic medicines were evaluated at 3 distinct dynamisations (30, 50 and 200 CH, where CH is (Centesimal-hahnemannian) as well as an untreated control and a chemical fungicide control (carbendazim 50 % WP), for a total of 9 treatments. The experiment was carried out *in vitro* utilising a CRD. In order to determine whether variations in potency levels would lead to distinct reactions in fungal development, the various dynamisations were chosen. *Calcarea carbonica, Thuja occidentalis, Silicea, Arnica montana,* Phosphorus, Sulfur and *Kali bromatum* were among the homoeopathic remedies assessed. In accordance with typical homoeopathic practices (18), all homoeopathic preparations were delivered as ready-to-use liquid potencies made in 70 % ethanol and acquired from a licensed homoeopathic pharmacy (SBL Global, New Delhi). Each treatment was conducted in triplicate. The untreated control consisted of culture medium without the addition of homoeopathic medicine, while the positive control treatment consisted of the addition of carbendazim 50 % WP at the recommended concentration. The experiment employed the phytopathogenic fungus *Sclerotinia sclerotiorum*, which was cultivated on PDA 39.5 g/L media. 20 mL of PDA medium was used to prepare 90 mm-diameter Petri dishes. For plates containing 20 mL of PDA, the volume of homoeopathic preparation was adjusted proportionally to maintain a 0.5 % (v/v) application, corresponding to 100 µL per plate (19). A Drigalsky loop was used to distribute the fluid uniformly across the medium surface. Each plate was infected at the center with a 5 mm diameter mycelial disc from an actively developing culture of *S. sclerotiorum* once the applied solution had completely dried. After this, the inoculated the plates were incubated at 19 ± 2 °C for 5–7 days. The percentage of inhibition was determined by assessing the fungal mycelial growth on the treated plates compared to the control, using Eqn. 1.

Results and Discussion

Pathogenicity test

After 1 week, inoculated stems developed water-soaked lesions that expanded, girdling the stem and spreading both upward and downward. White mycelial growth and sclerotia formation were later observed within dried tissues. The pathogen was re-isolated on PDA and identified microscopically as *S. sclerotiorum*, while control plants remained symptomless which is shown in Fig. 2. Based on morphological features, the fungus was identified as *S. sclerotiorum* (14, 15) which is shown in Fig. 3, 4.

Molecular identification

The isolate has been deposited in NCBI with accession number PX309100. This is the first report of *S. sclerotiorum* causing stem rot of ajwain in Bihar, India, a crop known to be highly susceptible to sclerotinia rot, with no resistant varieties reported (20–22). Similar results were also reported for *S. sclerotiorum* causing stem rot of tulsi (23) (Fig. 1).

Efficacy of homoeopathic treatments against *Sclerotinia sclerotiorum*

Significant variation in the suppression of *S. sclerotiorum* was seen in the *in vitro* evaluation of 9 treatments utilising homoeopathic medicines, including an untreated control and carbendazim 50 % WP as a positive control as shown in Fig. 5, Plate 1 and Table 1. Mycelial growth inhibition was highest in *Calcarea carbonica*

Fig. 2. Pathogenicity test of *Sclerotinia sclerotiorum* by cotton mycelium method. (A) Control; (B) attached cotton mycelium; (C) diseased plant.

Fig. 3. *Sclerotinia sclerotiorum* isolate SSR ajwain 2 developed on slant.

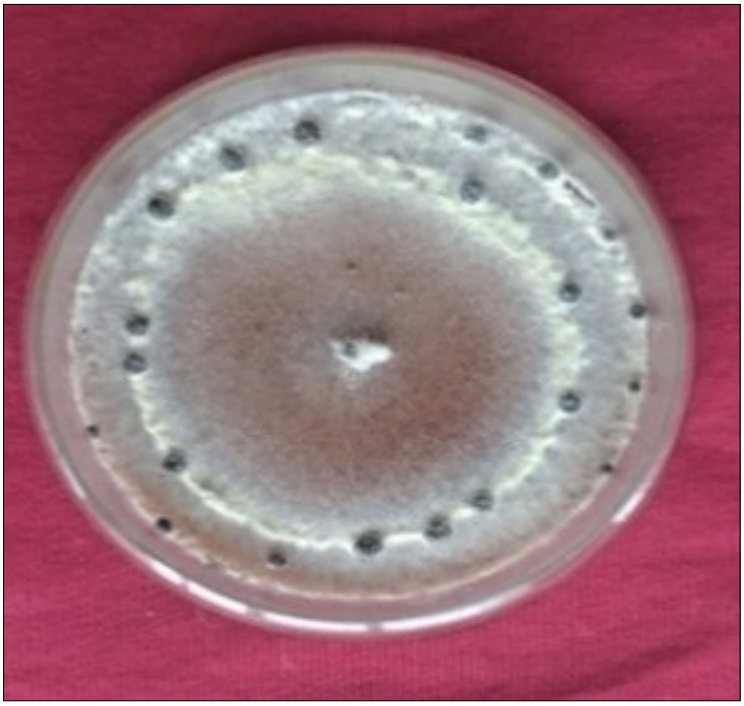


Fig. 4. *Sclerotinia sclerotiorum* isolate SSR Ajwain 2 (PX309100) growing on PDA with sclerotia developing at margin.

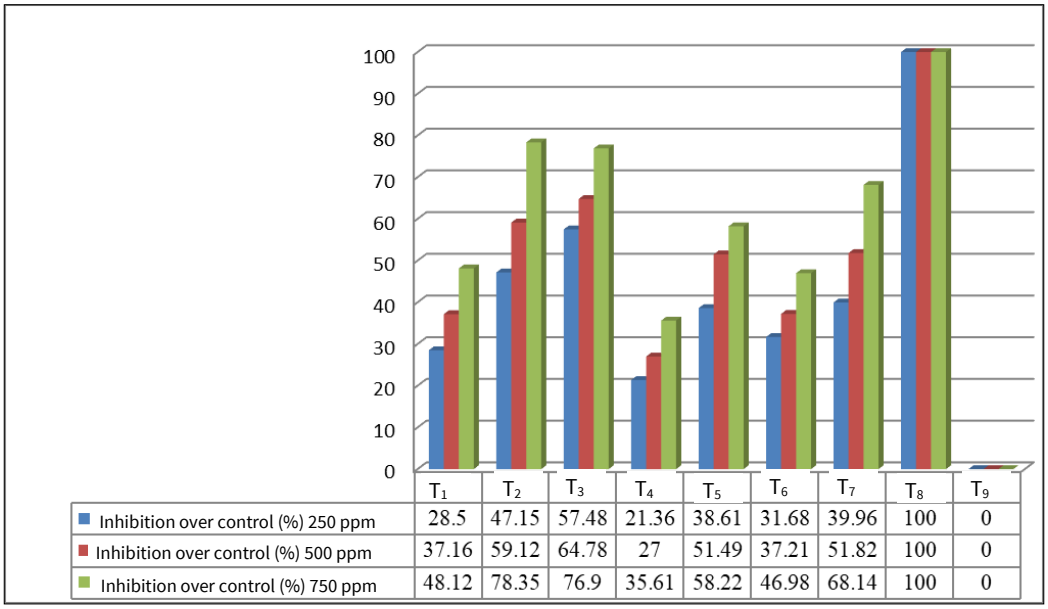


Fig. 5. *In vitro* effect of homoeopathic drugs against *Sclerotinia sclerotiorum* at different concentrations.

T_1: Phosphorus; T_2: *Calcarea carbonica*; T_3: *Thuja occidentalis*; T_4: *Kali bromatum*;T_5: *Arnica montana*; T_6: Sulfur; T_7: *Silicea*; T_8: carbendazim (standard check); T_9: control.

Table 1. *In vitro* effect of homoeopathic drugs against *Sclerotinia sclerotiorum* at different concentrations

S. No.	Homoeopathic drugs	Avg. colony diameter (mm)			Mycelium growth inhibition over control (%)		
		30 CH	50 CH	200 CH	30 CH	50 CH	200 CH
T_1	Phosphorus	64.35 (8.05)^c	56.55 (7.55)^c	46.69 (6.87)^c	28.50 (5.39)^f	37.16 (6.14)^f	48.12 (6.97)^f
T_2	*Calcarea carbonica*	38.26 (6.22)^h	31.69 (5.67)^g	19.48 (5.02)^f	57.48 (7.61)^b	64.78 (8.08)^b	78.35 (8.55)^c
T_3	*Thuja occidentalis*	47.56 (6.93)^g	36.78 (6.10)^f	20.79 (4.47)^g	47.15 (6.90)^c	59.12 (7.72)^c	72.57 (8.88)^b
T_4	*Kali bromatum*	70.77 (8.44)^b	65.69 (8.14)^b	57.95 (7.64)^b	21.36 (4.67)^g	27.00 (5.24)^g	35.61 (6.01)^g
T_5	*Arnica montana*	58.28 (7.67)^e	46.25 (6.84)^d	37.60 (6.17)^d	35.24 (5.98)^e	48.61 (7.01)^e	58.22 (7.66)^e
T_6	Sulfur	61.48 (7.87)^d	56.50 (7.55)^c	47.71 (6.94)^c	31.68 (5.67)^f	37.21 (6.14)^f	46.98 (6.89)^f
T_7	*Silicea*	54.03 (7.38)^f	40.59 (6.41)^e	28.67 (5.40)^e	39.96 (6.36)^d	54.90 (7.44)^d	68.14 (8.29)^d
T_8	Carbendazim 50% WP (Standard check)	0.00 (0.71)^i	0.00 (0.71)^h	0.00 (0.71)^h	100 (10.02)^a	100 (10.02)^a	100 (10.02)^a
T_9	Control	90.00 (9.51)^a	90.00 (9.51)^a	90.00 (9.51)^a	0.00 (0.71)^h	0.00 (0.71)^h	0.00 (0.71)^h
		SEM (±)	**CD (at 5 %)**	**CV (%)**			
	Factor A (Fungicides)	**0.02**	**0.01**	**1.24**			
	Factor B (Concentrations)	0.01	0.04	1.24			
	Factor A × Factor B	0.04	0.13	1.24			

Average of three replications.

Data within parentheses represents square root transformed value of corresponding data.

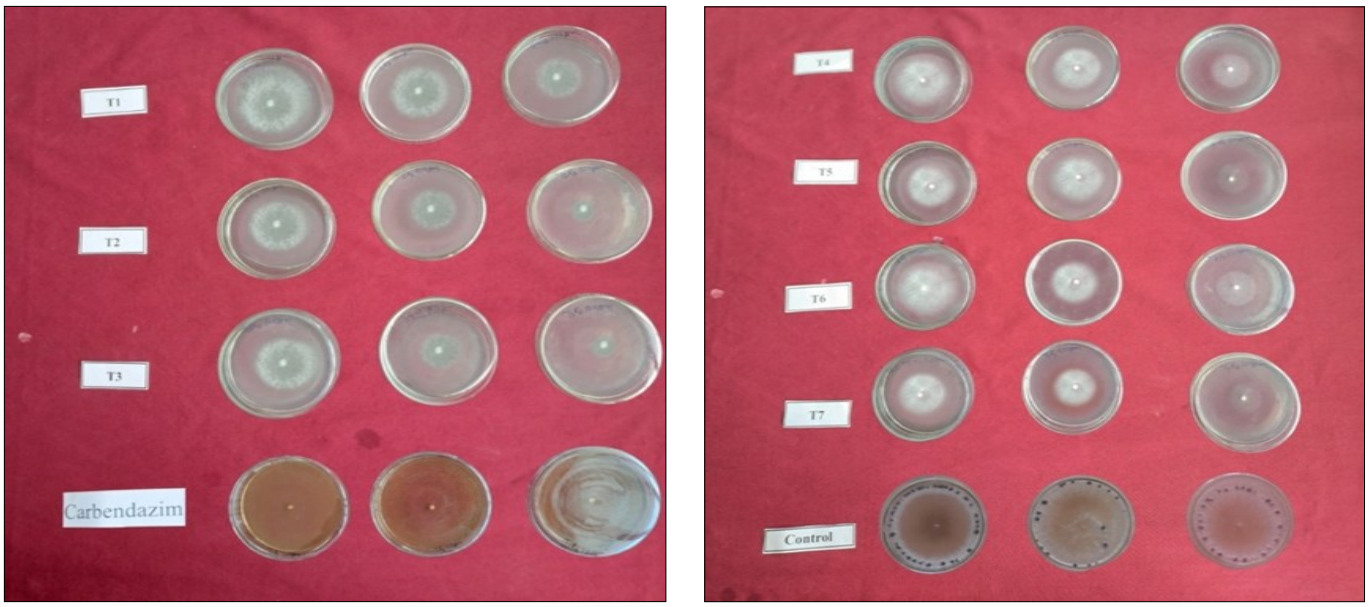


Plate 1. *In vitro* efficacy of homoeopathic drugs against *Sclerotinia sclerotiorum* at different concentrations (12th day).

(78.35 %), followed by *Thuja occidentalis* (72.57 %), *Silicea* (68.14 %), *Arnica montana* (58.22 %), Phosphorus (48.12 %), Sulfur (46.98 %) and *Kali bromatum* (35.61 %). These results are consistent with another study, which found that *T. occidentalis* and *Calcarea carbonica* exhibited the greatest inhibition at 250 and 500 ppm using varying potencies (24). The current investigation showed similar patterns at 200 CH, suggesting that potency affects antifungal activity. A previous study evaluated homoeopathic agents against multiple phytopathogens and reported 76.73 % inhibition by *T. occidentalis*, followed by *A. montana*, *Silicea* and Sulfur, which closely aligns with the inhibition pattern observed here (25). Likewise, another study reported a reduced white mold severity in beans using *Calcarea carbonica* and Phosphorus, although only Phosphorus remained effective at higher potencies (18). In the present study, Phosphorus (30 CH) caused 48.12 % inhibition, further indicating the role of potency and concentration. Studies have also reported complete inhibition of *Rhizoctonia bataticola* by *T. occidentalis* at higher concentrations, supporting its consistent antifungal potential across pathosystems (26, 27). Additionally, an earlier study demonstrated, up to 40 % inhibition of *S. sclerotiorum* by *Calcarea carbonica* at 1000 CH, which supports the higher inhibition (78.35 %) observed at 200 CH in the present study (28).

Comparative efficacy of chemical fungicides against *Sclerotinia sclerotiorum*

Seven number of commercial formulations of fungicides were tested *in vitro* at 50, 100 and 150 ppm to compare homoeopathic remedies. *Sclerotinia sclerotiorum* was completely inhibited (100 %) at 150 ppm by propiconazole 11.9 % + azoxystrobin 7.1 % SE, Tebuconazole 18.30 % + azoxystrobin 11 % SC and Difenoconazole 11.4 % + azoxystrobin 18.20 % SC. Azoxystrobin 23 % SC was the least effective (52.77 %), while propiconazole 25 % EC and Hexaconazole 5 % EC showed 96.13 % and 94.50 % inhibition, respectively which as shown in Table 2, Plate 2 and Fig. 6. Higher inhibition at 150 ppm suggests that fungicide combinations are more effective, most likely because of their synergistic multi-site activity.

These findings are consistent with earlier studies showing that carbendazim and thiophanate methyl completely inhibit *S. sclerotiorum* (29). Complete inhibition of *S. sclerotiorum* by carbendazim and mancozeb has also been documented (30). In addition, carbendazim has been shown to completely suppress mycelial growth and sclerotial formation (31). Carbendazim,

Table 2. *In vitro* effect of fungicides against *Sclerotinia sclerotiorum* at different concentrations

S. No.	Fungicides	Avg. colony diameter (mm)			Mycelium growth inhibition over control (%)		
		50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm
T_1	Hexaconazole 5 % EC	32.11 (5.71)^{c}	14.82 (3.91)^{c}	4.91 (2.33)^{c}	64.32 (8.05)^{f}	83.53 (9.17)^{d}	94.50 (9.75)^{b}
T_2	Azoxystrobin 23 % SC	81.33 (9.04)^{b}	71.54 (8.49)^{b}	42.5 (6.56)^{b}	9.63 (3.18)^{g}	20.51 (4.58)^{e}	52.77 (7.30)^{c}
T_3	Propiconazole 25 % EC	28.14 (5.35)^{d}	12.53 (3.61)^{d}	3.48 (1.99)^{d}	68.73 (8.32)^{e}	86.07 (9.30)^{c}	96.13 (9.83)^{b}
T_4	Propiconazole 11.9 % + Azoxystrobin 7.1 % w/w SE	8.61 (3.02)^{g}	0.96 (1.23)^{f}	0.00 (0.71)^{e}	90.43 (9.54)^{b}	98.90 (9.97)^{a}	100 (10.02)^{a}
T_5	Tebuconazole 18.30 % + Azoxystrobin 11 % w/w SC	12.40 (3.59)^{f}	1.01 (1.21)^{f}	0.00 (0.71)^{e}	86.22 (9.31)^{c}	98.80 (9.97)^{a}	100 (10.02)^{a}
T_6	Difenoconazole 11.4 % + Azoxystrobin 18.20 % w/w SC	17.22 (4.21)^{e}	6.47 (2.64)^{e}	0.00 (0.71)^{e}	80.86 (9.02)^{d}	92.81 (9.62)^{b}	100 (10.02)^{a}
T_7	Carbendazim 50 % WP (Standard check)	0.00 (0.71)^{h}	0.00 (0.71)^{g}	0.00 (0.71)^{e}	100 (10.02)^{a}	100 (10.02)^{a}	100 (10.02)^{a}
T_8	Control	90.00 (9.51)^{a}	90.00 (9.51)^{a}	90.00 (9.51)^{a}	0.00 (0.71)^{h}	0.00 (0.71)^{f}	0.00 (0.71)^{d}
		SEM (±)	**CD (at 5 %)**	**CV (%)**			
	Factor A (Fungicides)	**0.02**	**0.07**	**1.86**			
	Factor B (Concentrations)	0.01	0.04	1.86			
	Factor A × Factor B	0.04	0.12	1.86			

Average of three replications.

Data within parentheses represents square root transformed value of corresponding data.

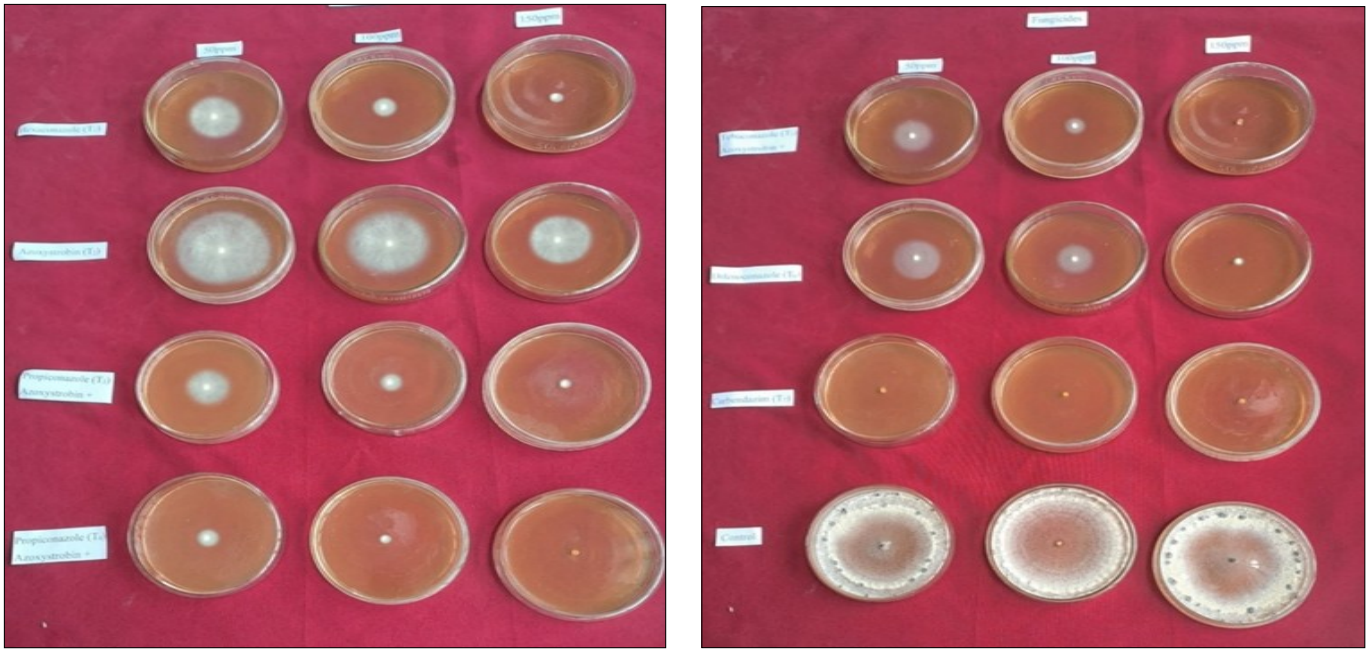
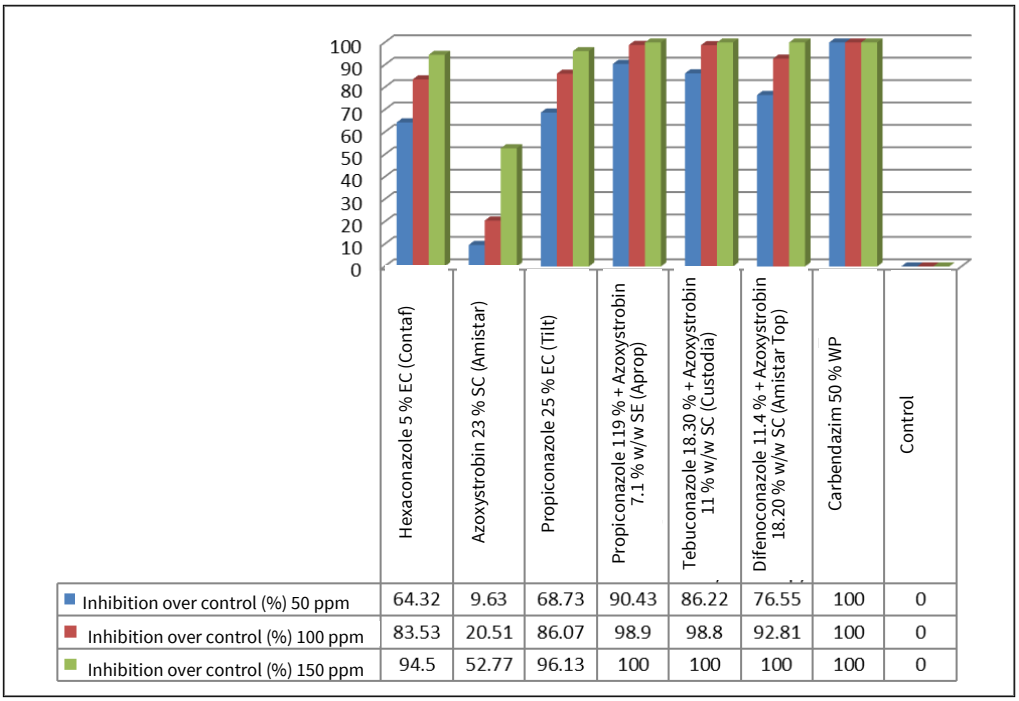


Plate 2. *In vitro* efficacy of fungicides against *Sclerotinia sclerotiorum* at different concentrations (12th day).



Inhibition over control (%) 50 ppm	64.32	9.63	68.73	90.43	86.22	76.55	100	0
Inhibition over control (%) 100 ppm	83.53	20.51	86.07	98.9	98.8	92.81	100	0
Inhibition over control (%) 150 ppm	94.5	52.77	96.13	100	100	100	100	0

Fig. 6. *In vitro* effect of fungicides against *Sclerotinia sclerotiorum* at different concentrations.

T_1: Hexaconazole; T_2: *A*zoxystrobin; T_3: Propiconazole; T_4: Propiconazole + Azoxystrobin; T_5: Tebuconazole + Azoxystrobin; T_6: Difenoconazole + Azoxystrobin; T_7: carbendazim; T_8: control.

thiophanate methyl, propiconazole, and hexaconazole have also been reported to be highly effective against chickpea stem rot (32). Complete suppression of the pathogen has been documented with propiconazole at higher dosages and with carbendazim and hexaconazole at 50 ppm (33). Carbendazim and carbendazim + mancozeb have been shown to suppress stem rot in coriander similarly (34). Additionally, other studies have reported that the consistent effectiveness of carbendazim and its combinations at 50–150 ppm in fennel and allied the crops (35, 36).

Conclusion

This study demonstrates that non-conventional disease management approaches can achieve biologically meaningful suppression of an aggressive necrotrophic pathogen under controlled conditions, with relevance extending beyond the specific crop-pathogen system examined. The results indicate that selected ultra-diluted biological formulations exhibit reproducible antifungal activity, partially validating the research hypothesis and questioning the exclusive dependence on high-dose synthetic fungicides for effective disease control. Although conventional fungicides remain superior in absolute efficacy, the performance of these formulations supports their potential inclusion within sustainable and integrated disease management strategies, particularly where residue-free production, environmental safety and reduced input costs are critical. From an industrial perspective, their low production cost, minimal ecological footprint and compatibility with organic and low-input farming systems suggest

conditional economic feasibility as complementary, rather than substitutive tools in future crop protection frameworks.

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Authors' contributions

BSR planned and conducted the entire research work, including laboratory experiments, data analysis, and preparation of the manuscript. AKM profound knowledge in plant disease diagnosis, fungal taxonomy, and disease management strategies provided the scientific foundation upon which this research was built. CSC guided the initial isolation and microscopic identification of the pathogen, to standardising pathogenicity tests. RP formulated effective management strategies under both *in vitro* conditions. DR timely feedback, critical evaluation of experimental data, and motivation during challenging phases greatly enriched the quality and depth of this work. RKC and AR participated in statistical analysis and helped in carrying out the design of the study. ANR also gave moral support throughout the journey. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Grammarly AI to improve the clarity, grammar, academic tone and overall professionalism of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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