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Total phenol and ortho-dihydroxy phenol content of chili as affected by *Fusarium solani* and its management

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Abstract. Chili (*Capsicum annuum* Miller) is an important spice and vegetable crop in Nepal and India, but its production is severely affected by a wilt disease caused by *Fusarium solani*, leading to 55–95% seedling mortality. The present study investigated changes in total phenols and ortho-dihydroxy phenols in healthy and *F. solani*–inoculated chili roots of different varieties at 2, 4, and 6 days after inoculation. Results showed a reduction in phenolic contents in susceptible varieties, whereas resistant varieties exhibited increased levels following pathogen infection. This indicates that resistant chili varieties possess an inducible defense mechanism involving enhanced synthesis of phenolic compounds and activation of defense-related enzymes, while susceptible varieties appear to be suppressive in this response. The study also evaluated management options for collar rot disease of chickpea caused by *Sclerotium rolfsii* through in vitro screening of plant extracts, biocontrol agents, and fungicides. Among the botanicals tested, the aqueous extract of *Acorus calamus* at 10% concentration showed the highest inhibition (80%) of mycelial growth of the pathogen. Extracts of *Agave americana* and *Allium sativum* also exhibited significant inhibitory effects, recording 68% and 67% inhibition, respectively. A concentration-dependent increase in antifungal activity was observed for all plant extracts. Based on these findings, effective integrated disease management strategies were developed for chickpea using neem cake, cow dung, *Acorus calamus* extract, the biocontrol agent *Trichoderma harzianum*, and 0.1% calixin. The integrated approach offers an eco-friendly and sustainable option for managing collar-rot disease in chickpeas.

Keywords: *Acorus calamus, Bio-control, Fungicides, Phenolic compounds, Sclerotium rolfsii*

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1. Introduction

Chili (*Capsicum annuum* Miller) is a vital horticultural crop globally, valued not only for its economic importance but also for its rich phytochemical content, particularly phenolic compounds that serve crucial roles in plant defense mechanisms (Torres-Rodriguez et al., 2025). Among these, total phenols and ortho-dihydroxy phenols are central to plant resistance responses, functioning as antioxidants and contributing to structural barriers against pathogen invasion. Biotic stresses, especially infections by soil-borne pathogens like *Fusarium solani*, disrupt normal physiological processes, resulting in reduced phenolic content and compromised plant health. *F. solani* is a devastating pathogen known to cause root rot and vascular wilt in chili, leading to significant yield losses (Abo-Elyousr et al., 2023). Recent studies have shown that pathogen attacks can modulate the phenolic profile of plants, often leading to either suppression or compensatory increases depending on the defense signaling pathways triggered. The exogenous application of elicitors such as salicylic acid (SA) and jasmonic acid (JA) has been demonstrated to boost phenolic accumulation and activate systemic resistance in *C. annuum* against fusarial wilt (Majeed et al., 2024).

Chili is one of the important spice cum-vegetable crops of Nepal and India. It is also called red pepper and is grown for its pungent fruits. Both green and ripe fruits are used to impart pungency to the food. As a condiment, it has become indispensable

in every Nepalese house. It is also used medicinally, in chutneys, and in pickles. About 25 pathogens have been reported to occur on chili. Among the diseases, Fusarium wilt, caused by *Fusarium solani*, is a devastating soil-borne disease of fungal origin (Maurya et al., 2008) that is gaining importance in other regions and has been recently observed in various parts of the country. The interaction between plants and their pathogens is complex and may be very specific to a given combination of the plant and the fungus. Many biochemical changes occur in plants after infection, and some of these have been associated with the expression of defenses that are activated (Chakraborty et al., 2005). The responses include the formation of lignin, the accumulation of cell-wall appositions such as papillae (Prats et al., 2005), and the early accumulation of phenols (Chakraborty et al., 1995) within host cell walls.

Conventional control of *F. solani* relies heavily on synthetic fungicides; however, these raise concerns over environmental toxicity and resistance development. Thus, sustainable and eco-friendly management strategies have gained momentum. Biological control agents such as *Bacillus subtilis* have shown promising antifungal effects against *Fusarium* spp. And can also enhance host defense by stimulating phenolic biosynthesis (Abo-Elyousr et al., 2023). Similarly, natural products like chitosan are recognized for their dual function: direct inhibition of fungal growth and activation of plant defense-related enzymes, including phenylalanine ammonia-lyase, which is involved in phenol synthesis (Torres-Rodriguez et al., 2025). In parallel, aqueous extracts from *Capsicum* species and other botanicals have demonstrated inhibitory effects on fungal pathogens due to their bioactive secondary metabolites, including flavonoids and phenols (Sepúlveda et al., 2024). Given the importance of phenolic compounds in mitigating pathogen impact, this study aims to assess changes in total phenol and ortho-dihydroxy phenol content in chili plants under *F. solani* stress and evaluate the effectiveness of biological and natural control agents in disease management and defense modulation.

2. Materials and Methods

2.1. Plant material, source of cultures and maintenance of stock culture

Three chickpea seed varieties were collected from agro-vates in Biratnagar for experimental use (Figure 1). A virulent culture of *Sclerotium rolfsii* Sacc., originally isolated from chickpea and confirmed as Sr-1 after fulfilling Koch's postulates, along with *Trichoderma harzianum* (Biocontrol agent), was obtained from the Immuno-phytopathology Laboratory, Department of Botany, North Bengal University. The fungus was sub-cultured on PDA slants and stored at 0 ºC, 20 ºC, and room temperature (28 ºC), with the pathogenicity of *S. rolfsii* tested at regular intervals to ensure virulence.

Figure 1. Culture pot

2.2. Inoculation technique of fungal pathogen and disease assessment

Sand–maize meal medium (3:1) was prepared as described by Chowdhury and Sinha (1995), inoculated with *Sclerotium rolfsii*, and incubated at 28 °C for 7 days. The resulting inoculums was thoroughly mixed with sterile soil in a 1:8 ratio, and 10 g of this fungus–soil mixture was incorporated into the top layer of soil in earthen pots containing chickpea seedlings to facilitate disease development (Chowdhury and Sinha, 1995). Disease assessment was performed through visual observation, and disease severity was recorded using a 0-6 rating scale at 2, 4, and 6 days post-inoculation. The scale was defined as follows: 0 = no visible symptoms; 1 = small lesions on lower leaves; 2 = spots on middle leaves with 10-20% browning; 3 = leaf blight with 20-40% of leaves dried and browning of the shoot; 4 = extensive spotting with 60-70% of leaves affected; 5 = severe withering and shoot

browning with about 80% of leaves affected; and 6 = complete plant death with upper withered leaves still attached (Chakraborty et al., 2016).

2.3. Extraction and estimation of phenolics

Phenols were extracted and quantified according to Mahadevan and Sridhar (1982) with slight modifications. Fresh tea root tissue (1 g) was cut into small pieces and immediately immersed in boiling absolute alcohol (4 mL/g tissue), then boiled in a water bath for 5-10 min. After cooling, the tissues were homogenized with 80% alcohol and filtered, and the residue was re-extracted for 3 min. The filtrates were pooled, the final volume was adjusted with 80% alcohol, and the extracts were stored at 4 °C in dark vials wrapped with brown paper to avoid light-induced degradation (Mahadevan & Sridhar, 1982). Total phenols were estimated using the Folin–Ciocalteau method. For each determination, 1 mL of the extract was mixed with 1 mL of Folin–Ciocalteau reagent and 2 mL of 20% Na_2CO_3, shaken thoroughly, and heated for 1 min in a boiling water bath. The mixture was then diluted to a final volume of 25 ml with double-distilled water. Absorbance was recorded at 650 nm using a Systronics photoelectric colorimeter (Model-101), and total phenol content was calculated using caffeic acid as the standard (Mahadevan & Ulaganathan, 1992). Ortho-dihydroxy phenols were estimated following the method of Mahadevan and Ulaganathan (1992). For each assay, 1 mL of the extract was mixed with 2 mL of 0.5 N HCl, followed by the addition of 1 mL of Arnow's reagent ($NaNO_3$ 10 g, Na_2MoO_4 10 g, distilled water 100 mL) and 2 mL of 1 N NaOH. The reaction mixture was then diluted to a final volume of 25 mL with distilled water and shaken thoroughly. Absorbance was measured at 515 nm, and ortho-dihydroxy phenol content was quantified using caffeic acid as the standard (Mahadevan & Ulaganathan, 1992).

2.4. Preparation of leaf extracts

Seven plant parts were screened *in vitro* against *Sclerotium rolfsii* to evaluate their effects on mycelial growth and sclerotial germination. Dried roots of *Acorus calamus* and *Agave americana*, bulbs of *Allium cepa* and *Allium sativum*, and leaves of *Lantana camara* and *Azadirachta indica* were processed following the method of Paul and Sharma (2002). For each treatment, 25 g of plant material was homogenized in 200 mL of sterile distilled water, filtered through muslin cloth, and centrifuged at 10,000 rpm for 20 min. The resulting clear supernatant was used as the crude extract (Paul & Sharma, 2002).

2.5. *In vitro* evaluation and foliar application

For *in vitro* antifungal assays, crude extracts were incorporated into PDA at 2.5, 5.0, and 10.0% concentrations, with PDA alone serving as control. Plates were inoculated with 6 mm discs from 6-day-old cultures of *S. rolfsii* and incubated at 25±3°C. Radial growth and spore formation were recorded after 5 and 15 days, and percent inhibition over control was calculated. In the broth assay, 5 mL of crude extract was mixed with 45 mL of sterilized PDB; controls received 5 mL of distilled water. Flasks were inoculated with 5 mm agar blocks of *S. rolfsii* and incubated at 28°C for 15 days, after which mycelia were harvested and dry weights determined. For foliar application, extracts were supplemented with Tween 80 and sprayed onto chickpea plants using a hand sprayer, while control plants received distilled water plus Tween 80.

2.6. Bio-control assay

The antagonistic activity of *Trichoderma harzianum* against *S. rolfsii* was tested using the dual plate method. Six-mm mycelial discs from 5-day-old cultures of both fungi were placed opposite each other on PDA plates (9 cm diameter) with 7 cm spacing. Three treatments were set up: (i) *S. rolfsii* inoculated 24 h before *T. harzianum*, (ii) *T. harzianum* inoculated 24 h before *S. rolfsii*, and (iii) simultaneous inoculation. Each treatment was replicated thrice and incubated at 28 ºC for 8 days, with appropriate controls. Colony diameter of *S. rolfsii* was measured, and inhibition zones were recorded.

2.7. Organic amendments

Cow dung, chicken manure, and goat manure (100 g each) were mixed with 1 kg soil and placed in earthen pots. Neem cake and oil cake were separately decomposed for one week in clay pots covered with polythene, after which 100 mL of the decomposed material was diluted with distilled water, and 10 mL was applied to the rhizosphere of each chickpea seedling prior to inoculation with *S. rolfsii*.

2.8. Fungicide treatment

Calixin (0.1%) mixed with Tween 80 in distilled water was sprayed on chickpea plants four times at 7-day intervals. Control plants received distilled water with Tween 80. Both treated and untreated plants were inoculated with *S. rolfsii* for disease assessment.

2.9. Evaluation of inducing agents

The effects of *Trichoderma harzianum* and *Acorus calamus* extract, alone and in combination with organic amendments and fungicide, were evaluated against collar rot of chickpea seedlings (ICC 3512) under pot culture conditions (Figure 1). *T. harzianum*, either alone or in combination with oil cake, chicken manure, and *A. calamus* extract, showed effective management. Combinations with organic additives provided greater protection than individual treatments, while integration of all three agents with fungicide (0.1% Calixin) gave the most effective control of *S. rolfsii*.

3. Results

3.1. Varietal resistance test

Artificial inoculation of three chickpea varieties with *Sclerotium rolfsii* resulted in clear varietal differences in disease response. Based on visual disease index scoring, BLACO SINALOA-92 exhibited the highest disease severity and was identified as the most susceptible variety. ICC 3512 showed the lowest disease index and was therefore classified as resistant, while ICC 3421 displayed intermediate disease symptoms and was considered moderately susceptible.

Biochemical analysis revealed significant variation in phenolic responses among the varieties following pathogen inoculation. Total phenol content in roots of susceptible varieties (ICC 3421 and BLACO SINALOA-92) declined progressively at 2, 4, and 6 days post-inoculation compared with their healthy controls. In contrast, the resistant variety ICC 3512 showed a marked and consistent increase in total phenol content after inoculation, indicating activation of host defense mechanisms.

A similar trend was observed for ortho-dihydroxy-phenols. Susceptible varieties exhibited a reduction in ortho-dihydroxy-phenol content following infection. However, ICC 3512 recorded the highest increase in ortho-dihydroxy phenols among the three varieties, suggesting a strong inducible defense response. Although BLACO SINALOA-92 and ICC 3421 showed some increase at later stages, the magnitude was considerably lower than that observed in ICC 3512 (Figure 2, A-F). These results indicate that higher phenolic accumulation is associated with resistance to *S. rolfsii* in chickpea.

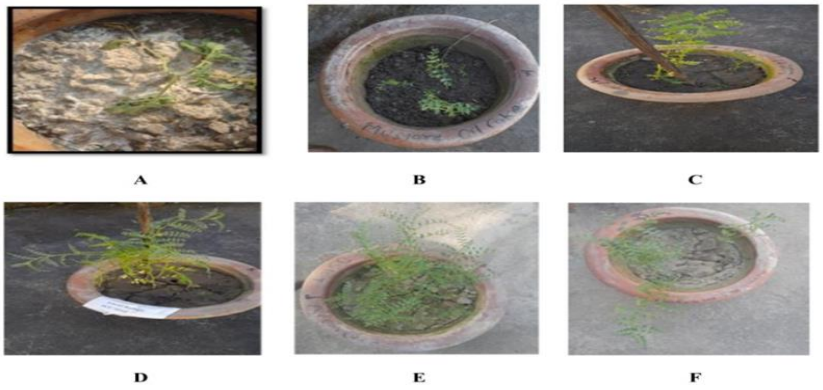

Figure 2. Chickpea plants following treatment with biocontrol agent and organic amendments. (A) Untreated and inoculated with *Sclerotium rolfsii*, (B) Untreated healthy, (C) Trichoderma viride inoculated with *S. rolfsii*, (D) Amended with cow dung manure, (E) Amended with chicken manure, (F) Amended with goat manure.

The 10% extract of *Acorus calamus* showed the highest inhibition of *S. rolfsii* mycelial growth (80%), with inhibition increasing alongside extract concentration. Extracts of *Agave americana* and *Allium sativum* also exhibited significant inhibition at 10% concentration (68% and 67%, respectively).

Disease index analysis indicated that all treatments significantly reduced disease severity compared to the untreated control (6.35±0.05). Individually, *Trichoderma harzianum* (2.63±0.03) and *A. calamus* root extract (3.50±0.04) showed moderate

suppression, while organic amendments (oil cake and chicken manure) were less effective (4.52±0.04). The combination of *T. harzianum*, *A. calamus* extract, and organic amendments further reduced the disease index to 1.55±0.05. The most effective control (0.74±0.04) was achieved when this combination was supplemented with Calixin (0.1%), demonstrating that integrated application of bio-control agents, botanicals, organics, and chemical fungicides provides superior management of collar rot. These results suggest that while biological and organic treatments are beneficial, their integration with a chemical fungicide enhances disease control, offering a sustainable and eco-friendly strategy for chickpea.

3.2. Bio-control agent

The antagonistic activity of *Trichoderma harzianum* against *Sclerotium rolfsii* was evaluated using the dual plate method. Mycelial discs (6 mm diameter) of the pathogen and antagonist were placed on opposite sides of PDA plates (9 cm diameter) with a 7 cm gap. Three treatments were tested: (1) *T. harzianum* inoculated 24 h before *S. rolfsii*, (2) *T. harzianum* inoculated 24 h after *S. rolfsii*, and (3) both inoculated simultaneously, with each treatment replicated three times and incubated at 28°C for 8 days alongside appropriate controls.

Observations of *S. rolfsii* colony diameter showed that maximum inhibition occurred when *T. harzianum* was inoculated 24 h prior to the pathogen. Inhibition was reduced when *T. harzianum* was applied 24h after *S. rolfsii*. When both were inoculated simultaneously, *T. harzianum* still effectively restricted radial growth of the pathogen. Overall, the antagonist consistently inhibited *S. rolfsii* across all treatments, with the highest efficacy observed in the pre-inoculation treatment (Figure 3, A-C).

After 8 days, *Trichoderma harzianum* overgrew and lysed *Sclerotium rolfsii*, while the pathogen's growth was halted and an inhibition zone was observed around it. Simultaneous inoculation of *T. harzianum* inhibited the pathogen's mycelial growth by 61.11%. Additionally, 25% culture filtrates of *T. harzianum* and *T. viride* reduced the mycelial weight of the sclerotial blight pathogen by 55.56% and 50%, respectively, after 5 days. These results indicate that both volatile and non-volatile extracellular metabolites of *Trichoderma* spp. contribute to growth inhibition of the pathogen.

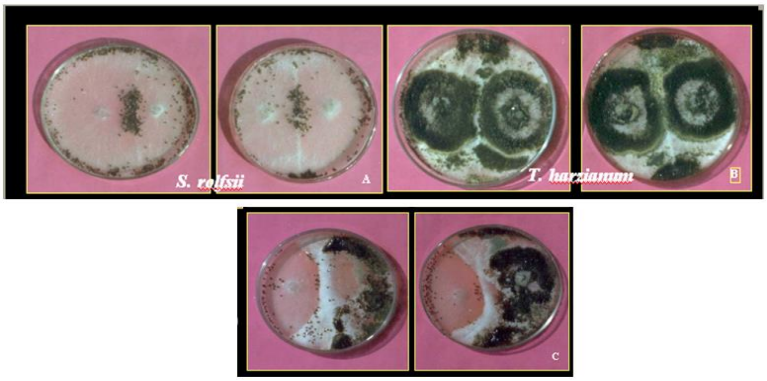

Figure 3 (A-C). Pairing of *S. rolfsii* with *Trichoderma harzianum*. (A). Homologous pairing of *S. rolfsii*; (B). *T. harzianum*, (C). Pairing of *S. rolfsii* with *T. harzianum*

3.3. *In vivo* evaluation

3.3.1. Growth promotion in chickpea seedlings

Chickpea seedlings of three varieties (ICC 3512, ICC 3421, and Blanco Sinaloa) were grown in soil amended with neem cake or oil cake, with each treatment consisting of 10 plants in triplicate (average of 30 plants). Observations were recorded at one- and two-month intervals following amendment and inoculation with *Sclerotium rolfsii*. Results showed that soil amendments with neem and oil cakes enhanced the growth of chickpea seedlings compared with pathogen-inoculated plants, and their growth approached or exceeded that of untreated, un-inoculated controls after two months. This indicates that neem and oil cake amendments positively influenced seedling growth and provided some protective effect against *S. rolfsii*.

3.3.2. Average of three replicates

Seedlings of two chickpea varieties (ICC 3512 and Blanco Sinaloa-92) were grown in soil amended with cow dung, goat manure, or chicken manure, with each treatment consisting of 10 plants in triplicate (average of 30 plants). Observations were

recorded at one- and two-month intervals following amendment and inoculation with *Sclerotium rolfsii*. Growth was higher in treated, uninoculated seedlings compared with treated, inoculated ones. Among the three organic amendments, cow dung produced the healthiest and most vigorous chickpea seedlings, outperforming goat manure and chicken manure (Figure 1).

3.3.3. Phenol content in chickpea varieties

Quantitative estimation of total phenol and ortho-dihydroxy phenol contents further confirmed the differential biochemical response among chickpea varieties. The resistant variety ICC 3512 exhibited a significant and progressive increase in total phenol content from 2 to 6 days after inoculation, reaching its maximum at 6 days. In contrast, susceptible varieties (BLACO SINALOA-92 and ICC 3421) showed a continuous decline in phenol levels compared to their respective controls. A similar pattern was observed for ortho-dihydroxy phenols, where ICC 3512 recorded a marked increase following pathogen challenge, whereas susceptible varieties exhibited a reduction throughout the observation period. These findings clearly indicate that enhanced accumulation of phenolic compounds is closely associated with host resistance against *Sclerotium rolfsii*, supporting the role of phenolics in induced defense mechanisms (Table 1).

Table 1. Changes in total phenol and ortho-dihydroxy phenol content in chickpea varieties following *Sclerotium rolfsii* inoculation

Variety	Treatment	Days after inoculation	Total phenol (mg/g)	Ortho-dihydroxy phenol (mg/g)
ICC 3512 (Resistant)	Control	2	2.45 ± 0.05	1.20 ± 0.03
		4	2.50 ± 0.04	1.22 ± 0.02
		6	2.48 ± 0.06	1.25 ± 0.03
	Inoculated	2	2.90 ± 0.06	1.50 ± 0.04
		4	3.25 ± 0.05	1.80 ± 0.05
		6	3.60 ± 0.07	2.10 ± 0.06
ICC 3421 (Moderate)	Control	2	2.30 ± 0.04	1.10 ± 0.02
		4	2.28 ± 0.05	1.08 ± 0.03
		6	2.25 ± 0.03	1.05 ± 0.02
	Inoculated	2	2.10 ± 0.05	0.95 ± 0.03
		4	1.95 ± 0.04	0.85 ± 0.04
		6	1.80 ± 0.06	0.78 ± 0.03
BLACO SINALOA-92 (Susceptible)	Control	2	2.20 ± 0.03	1.05 ± 0.02
		4	2.18 ± 0.04	1.02 ± 0.03
		6	2.15 ± 0.05	1.00 ± 0.02
	Inoculated	2	1.85 ± 0.04	0.80 ± 0.03
		4	1.60 ± 0.05	0.65 ± 0.02
		6	1.40 ± 0.04	0.55 ± 0.03

4. Discussion

In the present study, total phenol content decreased in susceptible chickpea varieties following inoculation with *Sclerotium rolfsii*, whereas resistant varieties showed an increase. Among the three tested varieties, ICC3512 exhibited the highest increase in total phenol levels post-inoculation. Similarly, ortho-dihydroxy phenol content declined in the susceptible varieties (ICC 3421 and Blanco Sinaloa 92) after infection with S. rolfsii. These findings align with those of Adandonon et al. (2017), who reported that phenolic content was higher in healthy cowpea seedlings compared to infected ones. Alterations in phenolic metabolism following fungal infection are well-documented and have been implicated in plant defense mechanisms (Mahadevan & Ulaganathan, 1992). Hammerschmidt and Nicholson (1977) observed distinct differences in phenol accumulation between resistant and susceptible maize varieties infected with *Colletotrichum graminicola*. Likewise, Sridhar and Ou (1974) reported variations in phenolic accumulation in rice infected with *Pyricularia oryzae*.

Among the seven plant extracts tested *in vitro* against *S. rolfsii*, *Acorus calamus* rhizome extract exhibited the highest inhibition of mycelial growth (80%), especially at a 10% concentration. Effectiveness increased with extract concentration. Other notable extracts included *Agave americana* (68%) and Allium sativum (67%). These results are consistent with

Mungkotnasawakul et al. (2002), who demonstrated the antifungal efficacy of *A. calamus* rhizome extract against Alternaria species. The antifungal activity is attributed to β- asarone, an active compound in *A. calamus* root, which inhibits mycelial growth and sclerotial production in *S. rolfsii*.

Further, studies have shown antifungal properties in various plant species. For instance, extracts of *Azadirachta indica* and *Catharanthus roseus* suppressed *S. rolfsii* at concentrations of 10%, 50%, and 100% (Bhagat, 2013), while Sravani et al. (2020) reported similar effects using extracts of Datura, lemongrass, onion bulb, and ginger rhizome at 10% and 15% concentrations. Additional research supports the antifungal efficacy of *Polyalthia longifolia* against *Macrophomina phaseolina* (Datar, 1999) and *Lantana camara* and *Ocimum sanctum* against *Drechslera sorokiniana*. Extracts from *Vinca rosea* (leaf, flower, stem, and root) have shown antifungal effects on *S. rolfsii*, *Fusarium oxysporum*, and *Aspergillus niger*, while *Lawsonia inermis* leaves have also demonstrated fungitoxicity.

In vivo experiments further revealed that *Trichoderma harzianum*, both alone and in combination with neem cake, oil cake, *A. calamus* root extract, and 0.1% calixin, provided complete control of collar rot disease in chickpea. Similar outcomes were reported by Gupta et al. (2005), who used *T. viride* in combination with neem oil, neem cake, and deodar needles, achieving total disease suppression. However, combinations involving cow dung, rabbit manure, and chicken manure were less effective. The most significant disease reduction was achieved using *T. harzianum* with neem cake, oil cake, *A. calamus* extract, and 0.0125% calixin.

Integrated disease management (IDM) has proven effective in various crops. Bhagat (2019) reported successful management of Fusarium wilt in tomato through the integration of bio-control agents, fungicides, organic amendments, and plant extracts. Tiwari and Mukhopadhyay (2003) demonstrated that seed treatment using carboxymethyl cellulose (CMC) with Gliocladium virens and vitavax offered up to 81.9% protection against root and collar rot in chickpea, along with increased seedling emergence, plant stand, and yield. Similarly, Upamanyu et al. (2002) found that T. viride showed the highest tolerance to carboxin, tebuconazole, and carbendazim among other biocontrol agents when used with oil cakes and fungicides under greenhouse and field conditions. The present findings confirm that integrating plant extracts, biocontrol agents, and fungicides through IDM strategies can effectively manage *S. rolfsii*-induced diseases in crops like chickpea and tomato.

5. Conclusions

The study highlights the pivotal role of phenolic compounds in the defense response of chili (*Capsicum annuum*) against *Fusarium* solani-induced wilt, demonstrating that resistant varieties can enhance phenolic synthesis following pathogen attack, while susceptible varieties show a decline. This suggests an inducible defense mechanism in resistant genotypes that could be utilized in breeding programs for disease resistance. Additionally, the in vitro evaluation of various treatments against *Sclerotium rolfsii* in chickpea revealed that Acorus calamus extract was most effective in inhibiting fungal growth, followed by *Agave americana* and *Allium sativum*. An integrated disease management strategy combining botanical extracts, bio-control agents, organic amendments, and fungicides proved effective, offering a promising and sustainable approach for managing collar rot in chickpea.

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