



RESEARCH ARTICLE

Effect of consortia of biocontrol agents and organic amendments in confronting collar rot (*Sclerotium rolfsii* Sacc.) disease in brinjal (*Solanum melongena* L.) for sustainable cultivation

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Abstract

Collar rot, caused by *S. rolfsii*, is a significant threat to brinjal cultivation in Tamil Nadu. The most virulent isolate of *S. rolfsii*, identified as IS (VIL)-9, exhibited the highest disease incidence (42.4 %) in field surveys and 92.56 % in pathogenicity tests of our previous studies. The *Bacillus* spp. and *Trichoderma* spp. exhibited antagonistic activity against *S. rolfsii*. Aqueous extracts of various oil cakes including neem, pungam, mahua, castor and cotton were prepared and tested for their antifungal efficacy against *S. rolfsii*. Among the oil cake extracts, neem oil cake (10 %) exhibited the highest inhibition of *S. rolfsii* growth (81.11 %), followed by mahua oil cake (76.66 %), pungam oil cake (57.77 %), castor oil cake (34.44 %) and cotton oil cake (18.88 %). In addition, a pot culture experiment was conducted to evaluate the effectiveness of combined applications of biocontrol agents and organic amendments against collar rot disease. Ten treatments were tested, including combinations of *Bacillus* sp., *Trichoderma* sp. and neem cake. *Bacillus* sp., *Trichoderma* sp. and neem oil cake are compatible with one another in the *in vitro* assay. Among the various integrated treatments, the combined application of both biocontrol agents, *Bacillus* sp. and *Trichoderma* sp., with neem cake (T₈) reduced the disease incidence to 14.59 %. The control pots with only *S. rolfsii* inoculation showed a disease incidence of 80.60 %. This study demonstrated that the integrated use of biocontrol agents and organic amendments such as neem cake and oil cake extracts significantly reduces the incidence of collar rot caused by *S. rolfsii* in brinjal cultivation providing an eco-friendly alternative to chemical control.

Keywords: *Bacillus* spp.; biocontrol agents; oil cake extracts; *S. rolfsii*; sustainable disease management; *Trichoderma* spp.

Introduction

Brinjal is a widely popular vegetable crop grown in India and other parts of the world. It is cultivated over an area of 728.00 kha, yielding 12660.00 kt annually, with a productivity of 17.7 t/ha (1). Brinjal is affected by numerous plant pathogens at various stages of its development. Among them, *S. rolfsii*, the causal agent of collar rot has emerged as a significant threat negatively impacting its production worldwide. Collar/foot rot can cause a 60-100 % mortality in eggplants (2). In recent years, biological control has gained importance in disease management. Biological agents and medicinal plant extracts have demonstrated strong inhibitory effects against *S. rolfsii*, making them eco-friendly and valuable for both the

environment and farmers (3). *Trichoderma harzianum* and *Trichoderma viride* exhibited strong antagonistic activity against *S. rolfsii* in sugar beet under both *in vitro* and *in vivo* conditions, with inhibition rates of 86 % and 88 % respectively. Similarly, *Trichoderma hamatum* effectively suppressed *S. rolfsii* by 79 % (4, 5). Moreover, the highest levels of glucanase, chitinase and peroxidase activities were observed in bulbs and roots of onion plants co-inoculated with *Trichoderma asperellum* and *S. rolfsii* (6). *Bacillus subtilis* controlled *S. rolfsii* in greenhouse-grown peanuts by 92 % (7). The antifungal compounds produced by *B. subtilis* exhibit strong antagonistic activity against *S. rolfsii* (8). *Bacillus megaterium* exhibited the highest mycelial growth inhibition against *S. rolfsii*; showed inhibition rates reaching 87.85 % (9). Seed treatment and soil

drenching with antagonistic cultures like *B. subtilis* significantly reduced seedling mortality in wheat by suppressing *S. rolfsii* (10). *Bacillus* isolates effectively inhibited the *S. rolfsii* through antibiosis (11). The exudation of extracellular compounds into the rhizosphere, production of antimicrobial substances and secretion of plant hormone helped to suppress plant diseases, promote growth and enhance nutrient absorption from the soil (12). *B. subtilis* strain G-1 successfully controls stem rot caused by *S. rolfsii* in groundnut through antibiotic production (13).

Among the organic amendments, mahua cake was found to be the most effective in reducing the incidence of sclerotium wilt in jasmine, followed by neem cake (14). An integrated management strategy against sclerotial wilt disease in bell pepper, demonstrating that the combined application of *T. viride*, chetu compound and neem cake was effective in controlling the disease (15). Neem cake as a soil amendment reduced stem rot disease in groundnut, showing the lowest incidence of disease (16). In the present study, an attempt was made to explore the antifungal activity of different oil cake extracts against the devastating pathogen *S. rolfsii* under *in vitro* conditions. Additionally, the combined application of biocontrol agents and oil cakes was tested against the pathogen under pot culture conditions.

Materials and Methods

Pathogen and effect of biocontrol agents

Different isolates of *S. rolfsii* from various brinjal-growing regions of Tamil Nadu were previously documented (17). Among them, IS(VIL)-9 exhibited the highest disease incidence in Vilangudi village [9.9498 °N, 78.0879 °E] in Madurai district of Tamil Nadu, recording 42.4 % during the survey and 92.56 % in pathogenicity tests. Additionally, we have studied the effect of *Bacillus* spp. and *Trichoderma* spp. on the growth of the collar rot pathogen *S. rolfsii* [Acc. No MT065836] under *in vitro* conditions. The *in vitro* efficacy tests revealed that the mycelial growth of *S. rolfsii* was significantly inhibited by the *Trichoderma* isolate T-AG, with an 84.44 % growth reduction, followed by the *Bacillus* isolate B-VI, which showed a 55.55 % growth reduction (17).

Preparation of aqueous extracts of oil cakes

An adequate quantity of each oil cake was powdered separately and soaked in sterile distilled water at a concentration of 1 g/mL water separately and left undisturbed overnight. The soaked substance was homogenized using pestle and mortar, which was then filtered with a muslin cloth. The collected filtrate was centrifuged at 10000 rpm for 15 min. The supernatant obtained was used as the standard oil cake extract solution (100 %) (18).

Effect of different oil cake extract against *S. rolfsii* through poisoned food technique

Five disparate organic amendments viz., neem cake, mahua cake, cotton cake, pungam cake and castor cake were assessed for the antifungal property against the virulent isolate IS (VIL)-9 of *S. rolfsii* use of poisoned food technique (19). The newly prepared PDA medium was poured into several conical flasks with a quantity of 40 mL for each conical flask. Aqueous extracts of each organic amendment were mixed at 10 mL with

40 mL of PDA medium to get 10 %, following sterilization of the medium in an autoclave. 15 mL of each sterilized PDA medium amended with extracts of organic amendments was poured into sterilized Petri plates separately and then it was allowed to solidify. A 9 mm mycelial disc of *S. rolfsii* was taken from an actively growing culture and placed at the centre of each Petri plate. It was then maintained at room temperature. The PDA medium without extracts of organic amendments was kept as control. Each treatment was replicated thrice. The mycelial growth of *S. rolfsii* was assessed after the full growth in the control plate (20).

D_c = average diameter of fungal growth (cm) in control

D_t = average diameter of fungal growth (cm) in treatment

Multiplication of inoculum in sand maize media

The inoculum of the unlike isolates of *S. rolfsii* was proliferated on sand maize medium separately. The medium constitutes 1900 g of sieved sand and 100 g of maize powder (19:1). The above two ingredients were blended and soaked in 400 mL of sterile distilled water per kg and put in glucose bottles. The bottles were sterilized at 121 °C with 15 psi pressure for 2 hr and for two alternate days, which then inoculated with two 9 mm mycelial disc of vibrantly growing *S. rolfsii* and observed after for 15 days at room temperature (28 ± 2 °C), until the production of brown coloured sclerotia which otherwise signal the maturity. The bottled compounds were mixed thoroughly ahead of applying to soil.

In vitro effect of combined application of antagonists and organic amendments

A pot culture experiment consisting of ten treatments was executed in the glasshouse at Department of Plant Pathology, Agricultural College and Research Institute, Madurai [9.9699 °N, 78.2040 °E] using two biocontrol agents viz., *Trichoderma* sp. and *Bacillus* sp., along with an organic amendment i.e., neem cake to evaluate the efficacy of application of biocontrol agents, organic amendment and their combinations against brinjal collar rot disease caused by *S. rolfsii*. These treatments were replicated thrice in completely randomized block design. Earthen pots were filled with sterilized potting medium (red soil:sand:FYM at 1:1:1 w/w/w) along with the inoculum of *S. rolfsii* at 5 g/pot. The antagonists and organic amendments were applied in four ways named, seed treatment, seedling root dip, soil drenching and soil application. The control pots were inoculated with pathogen alone. The effect of each treatment on the disease incidence was estimated individually.

Treatment details

The most promising isolates of antagonistic organism viz., *Bacillus* sp. B (VI)-9, *Trichoderma* sp. T (VT)-3 and organic amendment (neem cake) were administered in different interlude and combinations to fight the collar rot of brinjal in pot culture experiment. The study evaluated the various treatments for managing collar rot in brinjal viz., T₁- Seed treatment (ST) with *Bacillus* sp. @ 10 g/kg of seed + Seedling root dipping (SRD) of 30 - 35 days old brinjal seedlings with *Bacillus* sp. @ 10 g/L for 30 min; T₂- ST with *Trichoderma* sp. @ 4

g/kg of seed + SRD of 30 - 35 days old brinjal seedlings with *Trichoderma* sp. @ 4 g/L for 30 min; T₃- ST + SRD with *Bacillus* sp. + Soil application (SA) of neem cake @ 100 g/kg of soil; T₄- ST + SRD with *Trichoderma* sp. + SA of neem cake @ 100 g/kg of soil; T₅- T₃ + Soil drenching (SD) with *Bacillus* sp. @ 10 g/L at 30, 45 & 60 DAT (Days After Transplanting); T₆- T₄ + SD with *Trichoderma* sp. @ 10 g/L at 30, 45 & 60 DAT; T₇- ST (14 g/kg seed) + SRD @ 14 g/L + SA (15 g/kg of soil) with mixture of *Bacillus* sp. & *Trichoderma* sp. + SA of neem cake @ 100 g/kg of soil; T₇ + SD with mixture of *Bacillus* sp. & *Trichoderma* sp. @ 20 g/L at 30, 45 & 60 DAT; T₉- ST + SRD for 30 min + SD with carbendazim @ 0.2 % (positive check); T₁₀- Untreated control. Each treatment was three times replicated.

Statistical analysis

The data were statistically analysed using the AGRIS version 92 (21). The data were subjected to analysis of variance (ANOVA), the percentage values of the Disease Reduction Over Control and arcsine transformed values. Data were subjected to analysis of variance (ANOVA) at two significant levels ($p < 0.05$ and $p < 0.01$) and means were compared by Least significant difference (LSD).

Results and Discussion

The minimal mycelial growth of the pathogen was spotted in the neem cake amended plate with 1.70 cm and accounting to 81.11 % growth reduction over control (Table 1 & Fig. 1). The second least mycelia growth was noted in mahua cake with 2.10 cm recording growth reduction of 76.66 % over control. Cotton cake was found least effective in inhibiting the mycelial growth to 7.30 cm leading to 18.88 % growth reduction over control. Under *in vitro* conditions, groundnut oil cake and neem cake exhibited a suppression of 79.62 % and 76.29 % respectively, against collar rot and web blight of cowpea (25). Neem extract (*Azadirachta indica*) caused maximum inhibition of mycelial growth, sclerotial production, as well as sclerotial size and viability (26). Similarly, neem cake (10 %) extract treatment significantly reduced the oxalic acid content in the culture filtrate to 0.87 mg/mL, compared to 4.23 mg/mL in the untreated control, indicating its strong inhibitory effect on oxalic acid production by *S. rolfsii* (27).

Table 1. Efficacy of organic amendments against the mycelial growth of *S. rolfsii* *in vitro*

Sl. No.	Organic amendments	Concentration (10 %)	
		Mycelial growth (cm)*	Per cent growth reduction over control(%)
1.	Neem cake	1.70	81.11 (64.24)
2.	Mahua cake	2.10	76.66 (61.14)
3.	Pungam cake	3.80	57.77 (49.46)
4.	Castor cake	5.90	34.44 (35.93)
5.	Cotton cake	7.30	18.88 (25.75)
C	Control	9.00	00.00 (0.00)
CD (P=0.05)		0.24	1.87

*Mean of three replications

Values in the parentheses are arc sine transformed values

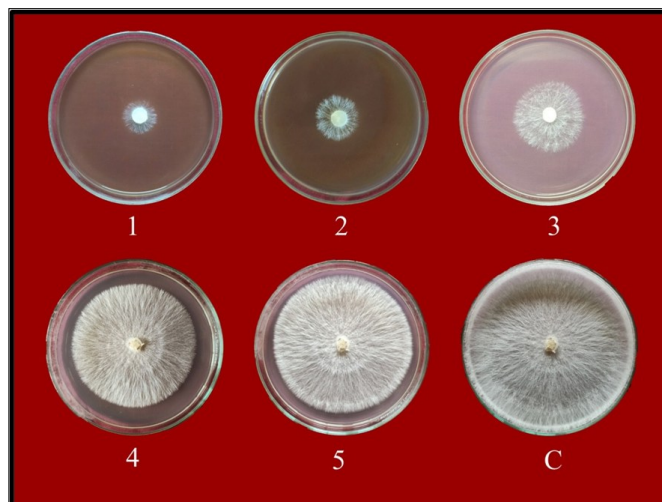


Fig. 1. Efficacy of extracts of organic amendments (10 %) on the mycelial growth of *S. rolfsii* (IS (VIL)-9) under *in vitro*. 1. Neem cake; 2. Mahua cake; 3. Pungam cake; 4. Castor cake; 5. Cotton cake; C. Control.

The *Trichoderma* spp. and *Bacillus* spp. antagonist isolated from native of brinjal showed that the mycelial growth of the *S. rolfsii* was significantly inhibited by the *Trichoderma* isolate T-AG with 84.44 % growth reduction followed by the *Bacillus* isolate B-VI with 55.55 % growth reduction. Similarly, *T. virens* NBAII Tvs12 antagonist showed buttressing mycoparasitism against *S. rolfsii* of groundnut by ceasing the growth to 87.91 % (22). *Trichoderma* completely overgrew the pathogen *S. rolfsii* with percentage inhibition rate of 81.36 % (23). Mycelium of *S. rolfsii* was decisively halted by five isolates of *Trichoderma* spp. (UD4-8, UD14-4, UD12-10, UD12-102 and KL10-12) with percentages of inhibition from 80-90 % (24). *Bacillus* strain LHSB1 was very beefy in clamping down on the radial growth of *S. rolfsii* hyphae to 93.8 % and 20 mm width inhibition zone. Strain LHSB1 formidably fettered sclerotia formation and germination, caused abnormalities and membrane integrity detriment to hyphae (28). Three antifungal lipopeptides viz., bacillomycin A, surfactin A and fengycin A, which were spotted in LHSB1 strain would be in responsible for the biocontrol activity of LHSB1 against *S. rolfsii* (28).

Effect of antagonists and organic amendments on collar rot of brinjal in pot culture

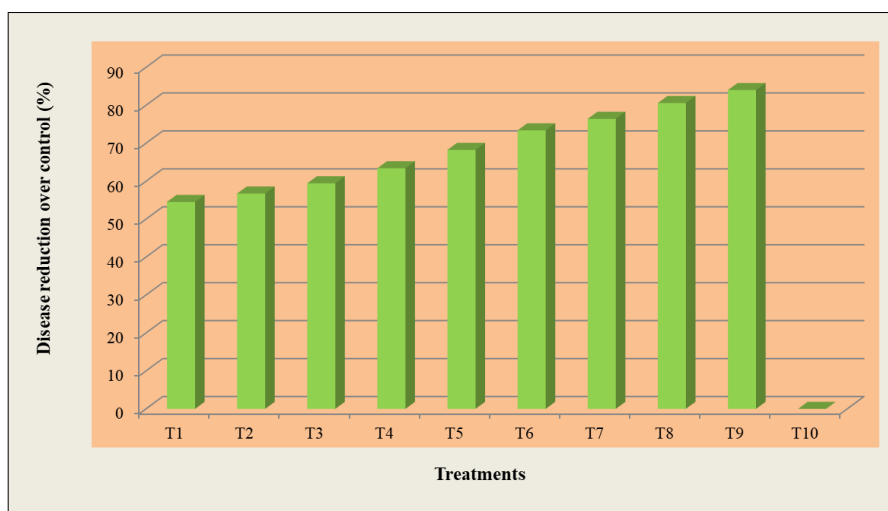
The experimental results revealed that the treatment T₈ resulted in the least disease incidence of 14.59 % over 75.23 % of untreated control which makes up for the highest disease reduction of 80.60 % (Table 2 & Fig. 2). The treatment T₇ was found to be second in controlling collar rot in pot culture. The treatment T₇ recorded 17.72 % disease incidence with 76.44 % growth reduction over control. The positive check T₉ (ST + SRD for 30 min + SD with carbendazim @ 0.2 %) was recorded 12.10 % disease incidence resulting in 84.03 % growth reduction over control. The treatment T₁ underperformed among all other treatments with the PDI (Per cent Disease Incidence) of 34.20 % and accounting to 54.33 % over control (Fig. 3). The treatment T₈ showed significant mean difference thus, outperforming all other treatments in this experiment. A similar observation was recorded with the soil application of *Trichoderma viride* Tv-Ve1 (5 g/pot) combined with neem cake (20 g/pot), which resulted in the lowest root rot disease incidence (5.09 %) and the highest disease reduction (93.53 %) over the control in coleus (29).

Table 2. Effect of antagonists and organic amendment against collar rot disease in brinjal plants under pot culture

T. No.	Treatments	Percent disease incidence(PDI)*	Disease reduction over control (%)
T ₁	ST with <i>Bacillus</i> sp. @ 10 g/kg of seed + SRD of 30 - 35 days old brinjal seedlings with <i>Bacillus</i> sp. @ 10 g/L for 30 min	34.20	54.53 (41.49)
T ₂	ST with <i>Trichoderma</i> sp. @ 4 g/kg of seed + SRD of 30 - 35 days old brinjal seedlings with <i>Trichoderma</i> sp. @ 4 g/L for 30 min	32.53	56.75 (42.77)
T ₃	ST + SRD with <i>Bacillus</i> sp. + SA of neem cake @ 100 g/kg of soil	30.50	59.45 (45.08)
T ₄	ST+ SRD with <i>Trichoderma</i> sp. + SA of neem cake @ 100 g/kg of soil	27.52	63.41 (47.35)
T ₅	T ₃ + SD with <i>Bacillus</i> sp. @ 10 g/L at 30, 45 & 60 DAT	23.88	68.25 (50.15)
T ₆	T ₄ + SD with <i>Trichoderma</i> sp. @ 10 g/L at 30, 45 & 60 DAT	20.01	73.40 (53.97)
T ₇	ST (14 g/kg seed) + SRD @ 14 g/L + SA (15 g/kg of soil) with mixture of <i>Bacillus</i> sp. & <i>Trichoderma</i> sp. + SA of neem cake @ 100 g/kg of soil	17.72	76.44 (55.84)
T ₈	T ₇ + SD with mixture of <i>Bacillus</i> sp. & <i>Trichoderma</i> sp. @ 20 g/L at 30, 45 & 60 DAT	14.59	80.60 (60.76)
T ₉	ST + SRD for 30 min + SD with carbendazim @ 0.2 % (positive check)	12.10	84.03 (65.49)
T ₁₀	Untreated control	75.23	00.00 (0.00)
CD (P=0.05)		1.30	2.52

Mean of three replications

Values in the parentheses are arc sine transformed values

**Fig. 2.** Pot culture experiment on the effect of antagonists and organic amendment on the collar rot disease of brinjal.**Fig. 3.** Effect of antagonists, organic amendment and their combination on the management of collar rot of brinjal under pot culture condition.

Application of *T. harzianum* inoculum to soil and seed dressing in the pots exhibited per cent disease reduction of 33-50 % and 72-83 % respectively (30). The application of FYM with seed treatment of *T. harzianum* at 4 g/kg seed + phosphate solubilizing bacteria at 25 g/kg seed resulted in a 26.0 % reduction in root rot of green gram caused by *Macrophomina phaseolina* (31). *S. rolfsii* is highly soil persistent; in this context, the different modes of application are very important for reducing the inoculum load of soil.

The seedling dip application of carboxin + thiram + *T. harzianum* + soil application of neem seed cake against collar rot of chilli resulted in the least mortality as well as the highest yield (32). A supportive result with the application of *Trichoderma* spp. and *Bacillus subtilis* as seed treatments and soil sprinkle with *Trichoderma* spp. had significantly higher effect on a disease incidence than *Bacillus* in cramping cowpea damping-off and stem rot (33). The tuber treatment of carboxin + *T. harzianum* along with soil application of FYM (Farm Yard Mannure) and neem cake, has reduced the wilt incidence provoked by *S. rolfsii* and increased the yield of potato (34).

Previous study reported similar results by the application of *Pseudomonas fluorescens* (Pf1) + *T. viride* Tv1 + *B. subtilis* Bs16 + neem cake + FYM + zinc sulfate, which hindered the collar and root rot disease incidence in physic nut (35). The soil application of *P. fluorescens* @ 20 g/pot and *B. subtilis* @ 25 g/pot had effectively reduced the wilt incidence of jasmine in pot culture experiment (36). Seeds pre-treated with *B. subtilis* subsp. *spizizenii*, *B. subtilis* subsp. *subtilis*, *Bacillus atrophaeus*, *Bacillus tequilensis* and *Streptomyces cyaneofuscatus* resulted in 50-58.5 % reduction in severity of root-rot of bean (37). In addition significant disease reduction of 56.67 %, 46.67 % and 26.67 % by *Bacillus* sp. 2PR9b, *Bacillus* sp. 1PR7a and *Bacillus* sp. 2P2, respectively, compared to the control (93.33 %) against the collar rot pathogen in tomato (38).

Neem seed cake, *Trichoderma* sp. and *Bacillus* sp. attributed the synergistic and additive growth effects that enhanced the plant vigour as well as substantial antagonistic activity of bio antagonist against collar rot pathogen. The addition of the oil cake into the soil increases the cation exchange capacity, water holding capacity as well as creates a good soil structure for plant growth and effectively reduces the harmful soil-borne pathogens. In the plant protection aspect, oil cake produces high antifungal principles against soil-borne pathogens like bacteria, fungi and nematodes.

Soil-borne diseases are effectively managed by organic amendments through various mechanisms. Mahua cake and neem cake demonstrated significant antimicrobial properties and effectively inhibited diseases. Notably, oil cakes rich in secondary metabolites, such as phenolic and flavonoid compounds, exhibited strong antimicrobial activity. Application of organic amendments alters the soil suppressiveness, stimulates the activities of beneficial microbiota such as *Rhizobacteria*, *Trichoderma* and *Pseudomonas* species, which create competition for nutrients with the infectious agents during decomposition and enhances the production of phenols or tannins with induced resistance in the plant system (39). Addition of neem cake into the soil enhances the population of *Trichoderma* spp.

Conclusion

The study demonstrated the effectiveness of *Trichoderma* spp. and *Bacillus* spp. in suppressing *S. rolfsii*, accentuated their potential as biocontrol agents. Among the oil cake extracts tested, some exhibited strong antifungal activity, further supporting their use in disease management. The combined application of biocontrol agents and organic amendments significantly reduced disease incidence in pot trials. These findings suggested that integrating biocontrol agents with organic amendments could serve as an eco-friendly and sustainable strategy for managing collar rot in brinjal.

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Authors' contributions

KK contributed to the writing of the original draft and conceptualization of the study. AA, MA, MT and JR were involved in revising the draft, including the incorporation of tables and figures and proofreading. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

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

Ethical issues: None

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