Biological Control 220 (2026) 106119

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Evaluation of bioagents and their SynCom inoculants for suppression of *Rhizoctonia bataticola* and *Sclerotium rolfsii* in chickpea: *In vivo* study

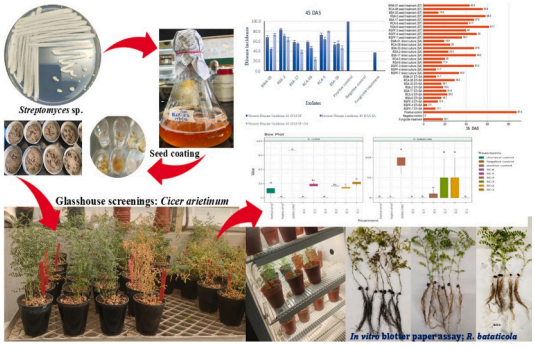
Syed Vahida Rehman [a,b], Rajan Sharma [b,*], Avula Vijaya Gopal [a], Gogisetty Sravani [b], Vadlamudi Srinivas [b], Nimmala Naresh [b], B. Sarvani [a], S. Gopalakrishnan [c], Mamta Sharma [b,**], D. Ramesh [a]

[a] *Acharya NG Ranga Agricultural University (ANGRAU), Lam, Guntur, AP 522034, India*
[b] *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad 502324, India*
[c] *International Institute of Tropical Agriculture (IITA), Dar es Salam, Tanzania*

HIGHLIGHTS

- Antagonistic microbes strongly suppressed *Sclerotium rolfsii* and *Rhizoctonia bataticola* in chickpea.
- In *in vitro* blotter paper assays, BWA-01, RCA-08, RSA-02 reduced the disease symptoms.
- Novel biocontrol isolate *Nesterenkonia aethiopica* BWA01 reduced *S. rolfsii* disease incidence.
- SynCom treatment (SC-6) significantly reduced the disease incidence up to 100% compared to control.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Antagonist
Biological control
*Pseudomonas*sp.
Soil borne
*Streptomyces*sp.
SynCom

ABSTRACT

The potential of antagonistic isolates was evaluated against the *Sclerotium rolfsii* and *Rhizoctonia bataticola* under glasshouse conditions over two seasons. *In vitro* blotter paper assay against *R. bataticola* revealed that three isolates (BWA01, RCA08 and RSA02) resulted in no root infection or disease symptoms, while three other isolates (BSA33, RCA5 and BSA17) decreased root infection to < 10% compared to 100% in control. In *in vivo* biocontrol studies (for two seasons/runs), isolates RSPF04 and BWA01 reduced the *S. rolfsii* incidence to below 20%, while RCA08 showed lowest disease incidence of 23.4% against *R. bataticola*. The key antagonistic actinomycetes were identified as *Streptomyces* spp. except isolate BWA01 as *Nesterenkonia aethiopica* and bacterial strains as *Pseudomonas* spp. through 16 s rRNA sequencing. Further, all the synergistic isolates were constructed into six synthetic communities (SynComs) and tested for their efficacy against both the pathogens, *S. rolfsii* and *R. bataticola*. SC-6 (SynCom with all potential isolates) treatment led to less prominent symptoms and significantly reduced the disease incidence up to 100% against both the pathogens. The remaining SynComs also significantly reduced the disease incidence. Our findings demonstrate the successful selection of antagonistic

* Corresponding author.
** Corresponding author.
E-mail addresses: rajan.sharma@icrisat.org (R. Sharma), mamta.sharma@icrisat.org (M. Sharma).

https://doi.org/10.1016/j.biocontrol.2026.106119
Received 24 January 2026; Received in revised form 26 May 2026; Accepted 30 July 2026
Available online 1 August 2026
1049-9644/© 2026 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

microbes including novel species like *Nesterenkonia aethiopica* and establish that their synergistic application in SynComs confers robust, reproducible protection against soil-borne fungal pathogens in chickpea.

1. Introduction

Chickpea (*Cicer arietinum* L.) stands as a cornerstone legume crop for the semi-arid tropics and ranks as the third most cultivated legume globally, after common bean and field pea. It is being cultivated across 50 countries (FAO 2025) and its global production reached 18.1 million tonnes from 15 million hectares in 2022–23. India is the world's leading producer, accounting for over 75% of this output with a production of 13.5 million tonnes in the 2023–24 season (Statista 2023). Over the past five decades, India's chickpea production has demonstrated significant growth, increasing from 5.2 to 11.9 million tonnes. Despite a remarkable production increase in India over the decades, chickpea cultivation is under threat and consistently limited by both biotic and abiotic stresses that reduce chickpea yields. However, in the face of rapidly changing climate, biotic stresses (majorly fungal pathogens) pose a far greater threat.

Among most pressing of the biotic threats is soil borne fungal pathogens, primarily *Rhizoctonia bataticola* and *Sclerotium rolfsii*. The fungus *R. bataticola* (Syn: *Macrophomina phaseolina*) has become a prominent threat to global chickpea production, causing dry root rot (DRR) disease in chickpea. Its severity is intrinsically linked to climate change, thriving well under drought conditions with optimal temperatures around 35℃ and can cause total crop loss (Gupta and Sharma 2015; Sharma et al. 2015; Rai et al. 2022). Similarly, *S. rolfsii* has become more prevalent in agricultural regions in recent years due to its competitive saprophytic survival ability. The fungus causes collar rot (CR), an emerging disease of chickpea, that can cause 55–95% mortality of chickpea seedlings under favourable conditions. The disease is more prevailing with increasing soil moisture and temperature in the inconsistent rainfall areas (Happenings 2019). The effective management of DRR and CR is challenging due to the pathogens' broad host range, which render crop rotation ineffective. Furthermore, the symptoms of DRR are severely exacerbated by the very abiotic stresses drought and high temperatures that are more common, creating a vicious cycle of infection and crop loss.

Consequently, there is a pressing need for sustainable control strategies. The use of microbial biocontrol agents represents one of the most promising and environmentally sound approaches. However, current research remains limited, having predominantly focused on *Trichoderma* spp. and its combinations with chemicals or plant derived biochemical extracts (Kumar et al. 2019). There is a critical gap in the exploration of a broader spectrum of bacterial and other microbial antagonists across diverse agro-climatic conditions (Gupta et al. 2021). Continued and diversified investigation into microbial biocontrol could yield more sustainable and effective biocontrol methods for managing soil-borne diseases. Therefore, the present study was planned to evaluate potential of biocontrol microbes that are antagonistic to collar rot and dry root rot diseases of chickpea in primary screening under *in vivo* glasshouse conditions.

2. Materials and methods

2.1. Bacterial isolates

Fourteen prominent antagonistic isolates selected were previously isolated from the chickpea rhizosphere and rhizoplane regions from different soils and locations randomly selected at the International Crops Research Institute for the Semi- Arid Tropics (ICRISAT) (17°.5′N latitude 78°.2′E longitude), Patancheru, Hyderabad and screened for their *in vitro* antagonistic and plant growth promoting activities. Table 1 reveals the details of prominent isolates and their *in vitro* activities.

Table 1
Details of prominent isolates obtained after *in vitro* screening.

Isolate	Isolated region	Type of soil	Stage of crop	Plant growth promoting activities	Effective against (dual culture assay)
BSA-14	Rhizosphere	Black soil	Physiological maturity phase	Sid, Ammo, β-1,3-gluc, Lip, HCN	*S. rolfsii*
BSA-17	Rhizoplane	Black soil	Physiological maturity phase	Sid, β-1,3-gluc, Lip, HCN	*S. rolfsii*
BSA-33	Rhizosphere	Black soil	Physiological maturity phase	IAA, Sid, Ammo, β-1,3-gluc, Chit, Lip, HCN, Zn, Si	*R. bataticola*
BWA-01	Rhizosphere	Black soil	Vegetative phase	Highest IAA, Sid, Ammo, Zn, β-1,3-gluc, Chit, Lip	Both
RCA-05	Rhizoplane	Red soil	Physiological maturity phase	IAA, Sid, Ammo, Zn, Si, β-1,3-gluc, Chit, HCN, Lip	Both
RCA-08	Rhizoplane	Red soil	Physiological maturity phase	Ammo, Zn, Si, β-1,3-gluc, Chit, Pro, Lip	*R. bataticola*
RSA-2	Rhizosphere	Red soil	Vegetative phase	Ammo, β-1,3-gluc, Chit, Lip, HCN	*S. rolfsii*
RSA-4	Rhizosphere	Red soil	Vegetative phase	IAA, Ammo, β-1,3-gluc, Chit, Lip, HCN	*R. bataticola*
RSA-5	Rhizosphere	Red soil	Vegetative phase	IAA, Sid, Ammo, β-1,3-gluc, Chit, Pro, Lip, HCN, Si	*S. rolfsii*
RSA-6	Rhizosphere	Red soil	Vegetative phase	IAA, Sid, Ammo, β-1,3-gluc, Chit, Lip, HCN	*R. bataticola*
RSPF-3	Rhizosphere	Red soil	Vegetative phase	IAA, Sid, β-1,3-gluc, Chit, Lip, HCN P, K, Si	*S. rolfsii*
RSPF-4	Rhizosphere	Red soil	Vegetative phase	IAA, Sid, β-1,3-gluc, Chit, Pro, Lip, HCN P, K, Si	*S. rolfsii*
RSPF-7	Rhizosphere	Red soil	Vegetative phase	IAA, β-1,3-gluc, Chit, Prot, Lip, HCN P, Si	*S. rolfsii*
RSPF-8	Rhizosphere	Red soil	Vegetative phase	IAA, Sid, β-1,3-gluc, Chit, HCN P, K, Si	*S. rolfsii*

Sid- siderophore; chit- chitinase, Lip- Lipase; β-1,3-gluc – β-1,3-glucanase; Pro- Protease; IAA- indole acetic acid; HCN– Hydrocyanic acid; P- Phosphorus; K- Potassium; Zn- Zinc; Si- Silica solubilization; Ammo- Ammonia production.
Note: All the isolates were obtained from chickpea rhizosphere and rhizoplane region.

2.2. Evaluation of isolates for in vivo plant growth promoting activities

Fourteen prominent antagonistic isolates with at least a few plant-growth promoting traits observed under *in vitro* evaluation were selected and evaluated for plant growth promotion in chickpea under glasshouse conditions. Soil mixture containing soil, sand and farmyard manure (3:2:1) was prepared and filled in plastic pots of 8″ dia. A total of 15 treatments (10 actinomycetes + 4 fluorescent *Pseudomonas* (RSPF-3,4,7,8 isolates) + uninoculated control) with three replicates were maintained. The seed treatment of ICCV-2 variety (standard/prominent variety) was done with lignite formulation of cultures for 45 min and 6 seeds were sown per pot later thinned to 3 seedlings per pot. *Pseudomonas* isolates were grown in sterile King's B broth media for 24 h, actinomycete isolates were grown in starch casein broth for 144 h. The 10 ml of well grown culture *i.e.*, 10^8 CFU/ml per plant was applied at regular intervals from the time of sowing, 7 days after sowing (DAS), 15 DAS, 30 DAS and 45 DAS. PGP traits including plant height, nodule number, nodule weight, root weight, shoot dry weight, root length, total plant weight were recorded at 30 DAS. At harvest, crop growth parameters including shoot weight, root weight, pod weight, harvest index and seed weight were recorded.

2.3. In-vitro blotter paper assay against R. bataticola under light chamber conditions

The antifungal activity of the test isolates was tested by blotter paper method (Pande et al. 2012; Sharma and Pande 2013; Gopalakrishnan et al. 2011). *R. bataticola* was cultured in potato dextrose (PD) broth at 28 ± 2°C for 7–10 days. The mycelial mats were removed and homogenized for 1–2 min to ensure uniform distribution of fungal mycelium or spores. Actinomycete isolates were grown in starch casein broth incubated at 28 ± 2°C for 7 days. The roots of two weeks old chickpea seedlings (of BG212 variety susceptible to dry root rot disease) were uprooted gently and washed with tap water to remove adhering sand particles and dipped in the pathogen (*R. bataticola*) inoculum kept in a beaker for about 30 min followed by dipping in test actinomycete isolate and the seedlings were placed side by side on blotter paper (45 × 25 cm) so that only roots were covered and the green tops of the seedlings remains outside the blotter paper. The blotter paper was moistened adequately and trays were incubated in light chamber at 28 ± 2°C for 8 days. Artificial light was provided for 12-h day length provided by fluorescent lights (120 μ mol m-2 s-1) and the blotter papers were moistened adequately on alternate day. The roots of the seedlings were observed for disease symptoms and recorded at the end of incubation. The experiment was replicated thrice. The disease onset was rated visually based on the growth of lesions on chickpea roots from 0 to 4 rating scale (Gopalakrishnan et al. 2011; Chandran et al. 2021) where 0 is no visible symptoms on roots (resistant), while 4 is highly susceptible with infected and completely discoloured roots.

2.4. Evaluation of potential isolates under glasshouse conditions against R. bataticola

Six potential antagonistic actinomycetes isolates (BWA-01, BSA-33, RCA-05, RCA-08, BSA-17 and RSA-2) against *R. bataticola* (Rb) identified from the *in vitro* studies were evaluated individually for their efficacy in pots for two seasons/runs. Each actinomycete isolate was inoculated by three different methods — M1 = seed treatment with actinomycete culture and sowing in potting mixture inoculated with *Rb* pathogen; M2 = soil application of actinomycete culture in potting mixture at the time of sowing (10 ml of well-grown actinomycete culture [10^8 CFU ml-1] was applied in soil) and M3 = seed treatment and soil application of actinomycete culture. Thus, the combination of actinomycetes isolates x three methods of inoculation constituted 18 independent treatments. Also, there were 3 more treatments as controls (Positive control – only pathogen treatment; Negative control – healthy uninoculated plants; and Chemical control – fungicide treated plants). The experiment had three replications. Seven days old *R. bataticola* culture, multiplied on sorghum seeds, was mixed with potting mixture (50 g per pot) and pots were maintained with 60% moisture at 35℃ temperatures to develop *Rb* inoculum in pots. Potting mixture was prepared by mixing Vertisols, sand, and farmyard manure at 3:2:1 (w/w) and was filled (1 kg) in 8′′ plastic pots (Hosco Horticultural Supplies Co, Mumbai, India) followed by inoculation with *Rb* one week before sowing. Chickpea seeds of BG212 variety susceptible to dry root rot disease were surface-sterilised (with 2.5% sodium hypochlorite solution in water for 5 min and rinsed 8 times with sterilised double distilled water) and treated with respective actinomycete isolates. Six inoculated seeds were sown (at 2—3 cm depth) in each pot. Plants were irrigated once every three days with 20 ml of sterilised distilled water. Fungicide used was Stump® (Carbendazim 12% + Mancozeb 63% WP) at 2 g/L. Incidence of dry root rot disease was recorded on 30 and 45 days after sowing (DAS). As there was increase in disease incidence at 45 days, disease incidence at 45 DAS was considered for comparison of treatments. Disease incidence was calculated using the method reported by Cao et al. (2011) with the following formula:

Disease incidence (%) = number of diseased plants/total number of plants *100.

2.5. Testing of the selected isolates for their antagonistic potential against S. rolfsii under glasshouse conditions

Seven promising actinomycetes isolates (BWA-01, BSA-33, RCA-05, RCA-08, BSA-17, RSA-2 and RSA-6) and three bacterial isolates (RSPF-3, RSPF-4, RSPF-7) identified from *in vitro* studies for their antagonistic activity against *S. rolfsii (Sr)* were further evaluated under glasshouse conditions for two seasons/runs. Each actinomycete isolate was tested using three different inoculation methods as described earlier i.e., 1. seed treatment, 2. soil application, and 3. seed treatment plus soil application of bioagents. This design resulted in a total of 30 distinct treatments (10 isolates × 3 inoculation methods). Additionally, three control treatments (Positive control, Negative control, Chemical control) were also included. The experiment had three replications.

Pot mixture was prepared as described above. Chickpea seeds of Annigeri variety susceptible to collar rot disease were surface-sterilised (with 2.5% sodium hypochlorite solution in water for 5 min and rinsed 8 times with sterilised double distilled water) and treated with respective test isolates. Fifteen days-old pathogen culture multiplied on sorghum seeds and groundnut shells was applied on upper layers of soil (50 g/pot) to 15 days old chickpea seedlings. The pots were maintained with adequate moisture at around 28℃ and left to develop symptoms. Plants were irrigated once every two days with 20 ml of sterilised distilled water. Fungicide used was Nativo® (Tebuconazole 50% + Trifloxystrobin 25% WG) at 0.5 g/L. Incidence of collar rot disease was recorded at 28 and 35 days after sowing (DAS).

2.6. Molecular characterization of prominent isolates

Pure cultures of selected prominent actinomycetes and fluorescent *Pseudomonas* isolates were subjected to 16S rDNA sequencing. Genomic DNA extraction of actinomycetes was done as per the protocols of Tripathi and Rawal (1998). In brief, actinomyces strains were inoculated in starch casein broth and incubated at 28℃ for 6 days. At the end of incubation, the cultures were centrifuged at 12,000 *g* for 5 min at 4℃ and the cells were washed with STE buffer (0.3 M sucrose, 25 mM Tris/HCl and 25 mM Na2EDTA, pH 8.0) twice. The cell pellets were resuspended in 400 μL STE buffer and 30 μL lysozyme (20 mg/ml STE buffer) followed by adding 40 μL of 10% SDS (w/v) and 8 μL of proteinase K and incubated at 60℃ for 10 min. At the end of incubation, 140 μL of 10% (w/v) CTAB in 0.7 M NaCl and 280 μL of 5 M NaCl was added with gentle mixing to avoid shearing of DNA and again incubated at 60℃ for 10 min. The lysate was extracted twice with 500 μL of chloroform/ isoamyl

alcohol (24:1) and centrifuged at 12,000 *g* for 10 min to collect the upper aqueous phase which was transferred to a fresh tube followed by addition of 300 μL isopropanol, 50 μL sodium acetate and then incubated at −20℃ overnight to pool out the DNA. At the end of incubation, the samples were centrifuged at 12,000 *g* for 5 min at 4℃. The supernatant was discarded, and DNA pellet was washed twice with 70 % (v/v) ethanol, vacuum dried and dissolved in 100 μL of TE buffer (10 mM Tris/HCl and 1 mM EDTA, pH 8.0).

DNA isolation of fluorescent *Pseudomonas* isolates was done as per the protocols of Green and Sambrook (2017). The fluorescent *Pseudomonas* strains grown in King's B broth for 24 h were transferred to 2 ml centrifuge tube and centrifuged at 12,000*g* for 5 min. Then supernatant was discarded, and pellet was dissolved in 400 μL of TE (100 mM Tris-Cl, 10 mM EDTA, pH 8.0). The pellet was vortexed twice for 20 sec each time. The bacterial lysate was treated with 50 μL of 10% SDS and 50 μL of proteinase K (20 mg/mL in TE, pH 7.5), followed by incubation for 1 h at 37C. After incubation, 500 μL of a 1:1 mixture of phenol:chloroform was added, and the samples were mixed gently by vortexing. The mixture was then centrifuged at 12,000g for 10 min. The supernatant was transferred to a fresh microcentrifuge tube and ~ 25 μL of 5 M NaCl and 1 mL of 95% ethanol were added. The tube was vortexed and centrifuged at 4C for 10 min, the supernatant was decanted, and pellet was air-dried for about 15 min at room temperature. The damp-dry pellet was finally dissolved in 100 μL of TE (pH 8.0).

The genomic DNA was handed over to genomics lab, ICRISAT for 16S rDNA sequencing. The genomic DNA was amplified using universal primers 27F (5′-AGAGTTTATCMTGGCTCAG- 3′) and 1492R (5′ −TACGGYTACCTTGTTACGACTT- 3′) and then sequenced. The obtained sequences were compared with similar sequences retrieved from GenBank (using the BLAST program and aligned using the Clustal W software) and the dendrogram was constructed by the neighbour-joining method (Saitou and Nei 1987; Altschul et al. 1990; Thompson et al. 1997). The sequences were submitted to NCBI and accession numbers obtained.

2.7. Compatibility screening of bacterial isolates

Plant growth-promoting rhizobacterial strains were tested for their compatibility by cross streak assay on nutrient agar medium used in multi-strain inoculation (Ankati et al. 2021). One bacterial strain was streaked on the solidified nutrient agar plate and incubated at 28 ± 2 °C for 24 h. The counter bacterial strain was streaked vertically to the growth of already streaked bacterial strain. The petri plates were replicated thrice and incubated for bacterial growth at 28 ± 2 °C for 48 h. The growth suppression of counter bacterial strain by already streaked bacterial strain were considered as noncompatible and mixing of bacterial growth of both strains were considered as compatible. All dual combinations of selected PGPR strains were evaluated for their compatibility to be used in multi-strain inoculation.

2.8. Designing the SynCom inoculants through functional screening approach

Criteria to design the SynCom inoculants through functional screening (bottom up) approach was based on Yin et al. 2022. The criteria followed in the present investigation was as follows.

different strains of the same genus and different genus (Phylogeny/Molecular identification).
relatively strong antifungal activities in single strain test.
with strong PGP and biocontrol traits.
one or two strains in a SynCom with strong antimicrobial activity of the metabolite produced.

A total of 11 actinomycetes, 4 fluorescence *Pseudomonas* strains, 2 *Rhizobium* strains (IC-59, and IC-76 collected from Biocontrol lab, ICRISAT) were selected to design the 6 SynComs of 6–8 members each. These SynComs consisted of 100%, 75%, 50%, 25%, or zero actinobacteria. SC-1 (only bacterial strains), SC-2 (50% actinomycetes sp.), SC-3 (75% actinomycetes sp.), SC-4 (100% actinomycetes sp.), SC-5 (25% actinomycetes sp.), SC-6 (all 17 strains).

2.9. Preparation of SynCom inoculants

Prior to mixing into SynComs, each bacterium was grown on a Petri dish with actinomycetes isolation agar (for actinomycetes) at 28 ℃ for 120 h and on king's B agar (for fluorescence *Pseudomonas* isolates) at 28 ℃ for 48 h. Each strain was individually grown in Bennett broth (BB) at 28 ℃ for 5–7 days until enough biomass was observed, and each strain was transferred to 4 ℃ for at least 24 h prior to mixing and inoculation to standardize pre-inoculation physiological conditions and minimize growth-rate differences of diverse microbes during inoculum preparation and to reduce metabolic stress, ensuring final community to be stable (Simmons 2020). To ensure that the starting inoculum contained approximately equal amounts of each community member, we normalized each strain by wet biomass. SynCom inoculants were prepared through mixing of equal volume of each strain (~10^8 cells/mL). Optical density (O.D.) of bacterial cultures (RSPF-3,4,7,8 and IC-59,76) was adjusted to 0.5 at 620 nm.

2.10. SynCom inoculants composition

SC-1. RSPF-03, RSPF-04, RSPF-07, RSPF-08 + two rhizobia strains (No actinomycetes).

SC-2. RSA-2, RCA-5, RSA-4, RSA-5, RSPF-03, RSPF-04, RSPF-07, RSPF-08 (50% actinomycetes).

SC-3. BWA-01, RCA-08, BSA-14, BSA-17, RSA-6, RSPF-7, RSPF-3 (75% actinomycetes).

SC-4. BWA-01, RCA-08, BSA-17, BSA-33, RCA-5, BSA-14, RSA-2. (100% actinomycetes).

SC-5. BWA-01, RCA-08, RSPF-03, RSPF-04, RSPF-07, RSPF-08, rhizobial strains (25% actinomycetes).

SC-6. BWA-01, RCA-8, BSA-17, RSA-2, RCA-5, BSA-14, RSA-4, RSA-5, RSA-6, BSA-4, BSA-33, RSPF-03, RSPF-04, RSPF-07, RSPF-08, two rhizobial strains (all strains).

2.11. Efficacy of SynCom inoculants for biocontrol of S. rolfsii and R. bataticola

Pot culture was conducted to evaluate the SynCom inoculants for biocontrol of *S. rolfsii* and *R. bataticola* in chickpea. Pot mixture was prepared by mixing Vertisols, sand and farmyard manure at 3:2:1 (w/w) and was filled (1 kg) in 8′′ plastic pots (Hosco Horticultural Supplies Co, Mumbai, India). Chickpea seeds of susceptible variety to collar rot and dry root rot disease were surface-sterilised (with 2.5% sodium hypochlorite solution in water for 5 min and rinsed 8 times with sterilised double distilled water) and treated with respective test synthetic inoculants; 10 ml of synthetic inoculants (10^8 CFU/ml) were applied through soil at 7 and 15 days after sowing. Ten days-old *S. rolfsii* pathogen culture multiplied on sorghum seeds was applied on upper layers of soil (50 g/pot) to chickpea seedlings of 10–15 days old. The pots were maintained with adequate moisture at around 28℃ and left for symptoms development. The experiment had three replications. Plants were irrigated once every two days with 20 ml of sterilised distilled water. Plants inoculated with only pathogen were treated as positive control. Fungicide used was Nativo® (Tebuconazole 50 % + Trifloxystrobin 25 % WG) at 0.5 g/L. Incidence of collar rot disease was recorded at 30 days after sowing (DAS).

In the second experiment, seven days old *R. bataticola* culture, multiplied on sorghum seeds, was mixed with pot mixture (50 g per pot) one week before sowing and pots were maintained with 60% moisture at 35℃ temperatures to develop *Rb* inoculum in pots. Seeds of susceptible

variety BG212 treated with synthetic inoculants were sown after one week of pathogen incubation and 10 ml of synthetic inoculants (10^8 CFU/ml) were applied through soil at 7 and 15 days after sowing. Plants were irrigated once every three days with 20 ml of sterilised distilled water. Plants inoculated with only pathogen were treated as positive control. Fungicide used was Stump® (Carbendazim 12 % + Mancozeb 63 % WP) at 2 g/L. The experiment had three replications. Disease symptoms and incidence of dry root rot disease was recorded on 45 days after sowing. Percent disease incidence for both the diseases was calculated.

2.12. Statistical analysis

All the experiments were statistically analyzed by ANOVA using GenStat 10.1 version (Fisher 1918). All the experiments were performed in triplicates and mean values at 5 % statistical significance were compared. Tukey's test was used to statistically assess significant differences between treatments at 5% level of significance (Tukey 1977). The figures were generated using R software (version 4.4.3).

3. Results

3.1. PGP activities of prominent isolates under glasshouse conditions

Under glasshouse conditions at 30 DAS, the selected isolates exhibited varied effects on different growth parameters, with some significantly enhancing the nodule weight (up to 78%), nodule number (86%), root weight (41%) over the uninoculated control (Table 2). Whereas there was slight enhancement in shoot weight (18%), root length (20%) and in plant height at 30 DAS. At harvest, we observed significant increase in the harvest index (68%), shoot weight (34.8%), and seed weight (85%) compared to control (Table 3). Of all the isolates, BWA-01, RCA-5, RCA-8, RSPF-3, 4, 7 demonstrated significant improvements in PGP traits with positive impacts on shoot weight, nodule number, nodule weight, root weight, seed weight, harvest index, ultimately enhancing both productivity and yield efficiency.

3.2. In vitro blotter paper assay

Disease screening of potential isolates that were antagonistic to *R. bataticola* in dual culture assay was done by blotter paper assay (Fig. 1). There were significant ($p < 0.05$) differences among the treatments. The treatment with only pathogen culture (positive control) recorded complete root infection (rating 4) with a disease incidence of 100.0% at the end of the incubation period. Among all bacterial culture treatments, three strains BWA-01, RCA-08 and RSA-2 resulted in no disease symptoms i.e., zero disease incidence (score 0 on 0–4 scale). Whereas three treatments, BSA-33 (3.3%), RCA-05 (6.7%) and BSA-17 (6.7%) resulted in infection rate < 10% with very few small lesions on roots (rating 1–2). Clear lesions on roots were observed in the remaining treatments (score 3–4) with an infection rate ranging from 16.7% to 63.3% (Fig. 2).

Table 2
Effect of prominent isolates on crop growth parameters of chickpea under glasshouse conditions at 30 DAS.

Isolate	Plant height (cm)	Nodule numbers (plant^{-1})	Nodule weight (mg/ plant)	Root length (cm)	Root weight (g/ plant)	Shoot weight (g/ plant)
BSA-14	35.67^{a}	26.67^{b}	12.97^{f}	18.27^{b}	0.270^{c-e}	1.44^{d}
BSA-17	39.00^{a}	34.33^{a}	11.60^{g}	18.77^{bc}	0.329^{a-c}	1.90^{ab}
BSA-33	39.67^{a}	31.33^{a}	10.47^{i}	11.23^{c}	0.314^{a-d}	1.72^{bc}
BWA-01	36.00^{a}	40.33^{a}	19.53^{a}	25.50 ^{a}	0.331^{a-c}	2.01^{a}
RCA-5	38.67^{a}	33.67^{a}	8.97^{j}	21.30^{ab}	0.353^{ab}	2.01^{a}
RCA-8	37.33^{a}	33.00^{a}	13.57^{e}	20.17^{ab}	0.387^{a}	1.79^{a-c}
RSA-2	38.67^{a}	24.33^{b}	17.60^{b}	20.87^{ab}	0.251^{c-e}	1.70^{bc}
RSA-4	39.00^{a}	38.00^{a}	15.93^{c}	20.70^{ab}	0.305^{b-d}	1.64^{cd}
RSA-5	22.67^{b}	35.67^{a}	13.77^{d}	20.13^{ab}	0.318^{a-d}	1.74^{bc}
RSA-6	38.33^{a}	27.33^{b}	6.77^{k}	15.67^{bc}	0.291^{b-e}	1.69^{b-d}
RSPF-3	37.33^{a}	49.33^{a}	5.93^{l}	17.10^{bc}	0.278^{c-e}	1.85^{a-c}
RSPF-4	42.33^{a}	23.00^{b}	3.27^{n}	16.33^{bc}	0.228^{e}	1.88^{a-c}
RSPF-7	40.67^{a}	43.33^{a}	3.07^{o}	21.67^{ab}	0.248^{de}	1.88 $^{a-c}$
RSPF-8	40.33^{a}	21.33^{b}	5.53^{m}	18.83^{bc}	0.254^{de}	1.84^{a-c}
Control	38.67^{a}	16.00^{b}	10.97^{h}	18.67^{bc}	0.204^{c-e}	1.69^{b-d}
SE(d)	4.09	10.64	0.007	4.117	0.04	0.12
SE(m)	2.89	7.53	0.005	2.911	0.03	0.09
LSD ($P \leq 0.05$)	8.36	21.8	0.015	8.43	0.07	0.25

Table 3
Effect of prominent isolates on chickpea crop growth parameters under glass house conditions at harvest.

Isolate	Harvest index (%)	Pod weight (g/ plant)	Seed weight (g/ plant)	Shoot weight (g/plant)
BSA-14	16.03^{b-d}	0.965 $^{d-f}$	0.777^{b-d}	4.07^{b}
BSA-17	21.83^{a-d}	1.267^{c-f}	0.918^{b-d}	3.25^{b}
BSA-33	22.22^{a-d}	1.194^{c-f}	0.974^{b-d}	3.41^{b}
BWA-01	32.38^{a}	2.416^{a}	1.932^{a}	4.05^{b}
RCA-5	32.84^{a}	1.807^{a-c}	1.462^{ab}	3.08^{c}
RCA-8	27.13^{ab}	1.665 $^{a-d}$	1.330^{a-c}	3.54^{b}
RSA-2	21.12^{a-d}	1.251^{c-f}	0.994^{b-d}	3.65^{b}
RSA-4	28.98^{ab}	1.721^{a-d}	1.396^{a-c}	3.51^{b}
RSA-5	24.16^{a-c}	1.522^{b-e}	1.232^{a-c}	3.82^{b}
RSA-6	20.49 $^{a-d}$	1.289^{c-f}	1.053^{b-d}	4.06^{b}
RSPF-3	19.97^{a-d}	1.381^{c-e}	1.171^{bc}	4.68^{a}
RSPF-4	11.92^{cd}	0.844^{ef}	0.707^{cd}	5.37^{a}
RSPF-7	8.33^{d}	0.817^{f}	0.682^{d}	4.61^{a}
RSPF-8	14.95^{b-d}	1.059^{c-f}	0.852^{b-d}	4.81^{a}
Control	19.55^{a-d}	2.181^{ab}	1.045^{b-d}	3.98^{b}
SE(d)	6.91	0.38	0.35	0.71
SE(m)	4.88	0.27	0.25	0.50
LSD ($P \leq 0.05$)	14.1	0.78	0.72	0.91

3.3. Efficacy of potential isolates against R. bataticola under glasshouse conditions

Six potential isolates (BSA-17, RCA-08, BSA-33, RCA-5, BWA-01 and RSA-2) were selected for pot experiments to assess their efficacy against *R. bataticola* based on their *in vitro* antagonistic screening, PGPR activities, and antifungal activity assessed through blotter paper assay. The results showed inconsistent response against the pathogen in different methods of treatment, but there were significant differences among the isolates ($p < 0.05$). The treatment with only pathogen (positive control) recorded the disease incidence of 83.4% at 30 DAS which increased to 100% at 45 DAS. In the seed treatment method, three isolates RCA-08, BSA-33 and BSA-17 could reduce the disease incidence by up to 40% over control at 45 DAS. Fungicide control recorded the disease incidence of 37.8% at 45 DAS. In direct culture treatment with bacterial isolates (soil application), the range of disease incidence was 44.5% – 70.9% with two isolates BWA-01 (44.5%) and RCA-8 (46.7%) exhibiting less than 50% disease incidence compared to 100% in positive control. In the third method of application, (seed treatment + soil application), disease incidence ranged from 23.4% to 79.5% at 45 DAS. RCA-08 recorded lowest disease incidence of 23.4%, followed by BSA-17 (38.6%) and BSA-33 (46.7%) (Fig. 4). Overall, RCA-08 and BSA-17 were identified as potential bioagents to reduce the disease severity by more than 60% over control.

3.4. Efficacy of potential isolates against S. rolfsii under glasshouse conditions

Ten potential isolates were screened for their efficacy against *S. rolfsii* under glass house conditions. The results indicated that seed treatment



Fig. 1. Disease screening of potential isolates for *in vivo* antifungal activity against *R. bataticola* pathogen through blotter paper assay. *Positive control (only pathogen treated); Negative control (without any culture treatment)*.

combined with soil application of bacterial treatments potentially reduced the disease incidence of *S. rolfsii* to 3.3% to 29.2% in chickpea seedlings compared to other treatments (pooled data of two seasons). Low disease incidence of less than 20% was observed in most of the isolates (BWA-01, BSA-33, RSA-2, RSA-6, RSPF-03, RSPF-04, RSPF-07) in this method of inoculation at 35 days after sowing (DAS) compared to 61% disease incidence in positive control. Statistical analysis indicated a significant difference ($p < 0.05$) among the treatments. Initially, at 10 days after inoculation of pathogen, positive control i.e., the chickpea seedlings inoculated with only pathogen, showed the disease incidence of 61.1% which further increased to 87.5% at 35 DAS (Fig. 3). Among test isolates, highest disease incidence of 61.7% was recorded in RSA-6 at 35 DAS. The lowest disease incidence in only seed treatment was observed in BSA-33 (13.9%) compared to 87.5% in positive control. Fungicide treated control recorded the disease incidence of 18.1%. Direct culture treated ones showed the disease incidence ranging from 11.7% to 49.2% at 35 DAS with 45–80% reduction in severity. Lowest disease incidence was observed in RSPF-04 (11.7%), followed by RSA-6 (17.2%), BWA-01 (19.4%), RCA-5 (20.0%), RSA-2 (23.3%) and RCA-08 (25.0%). Overall, RSPF-04, followed by BWA-01 were found to be promising and recorded lowest disease incidence.

3.5. Molecular characterization of potential isolates

The 16S rDNA sequencing of potential isolates, BWA-01, RCA-08, BSA-17, RCA-5, BSA-33, RSPF-04 and RSPF-7 indicated that all the actinomycete isolates belong to *Streptomyces* genus except BWA01 which was identified as *Nesterenkonia* sp., and the bacterial isolates belonged to *Pseudomonas* spp. (Table 4). Potential isolates were identified as *Nesterenkonia aethiopica* BWA01, *Streptomyces rocheii* BSA33, *Streptomyces sampsonii* BSA17, *Streptomyces champavati* RCA5, *Streptomyces harbiensis* RCA08, *Pseudomonas putida* RS4 and *Pseudomonas*

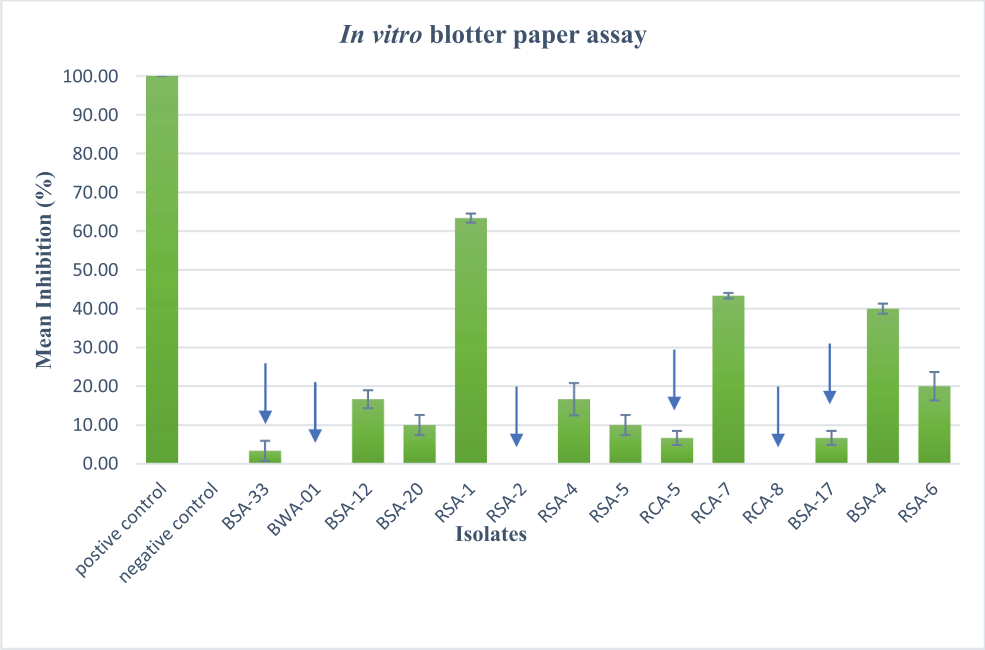


Fig. 2. *In vivo* antifungal activity of prominent isolates against *R. bataticola* pathogen through blotter paper assay.

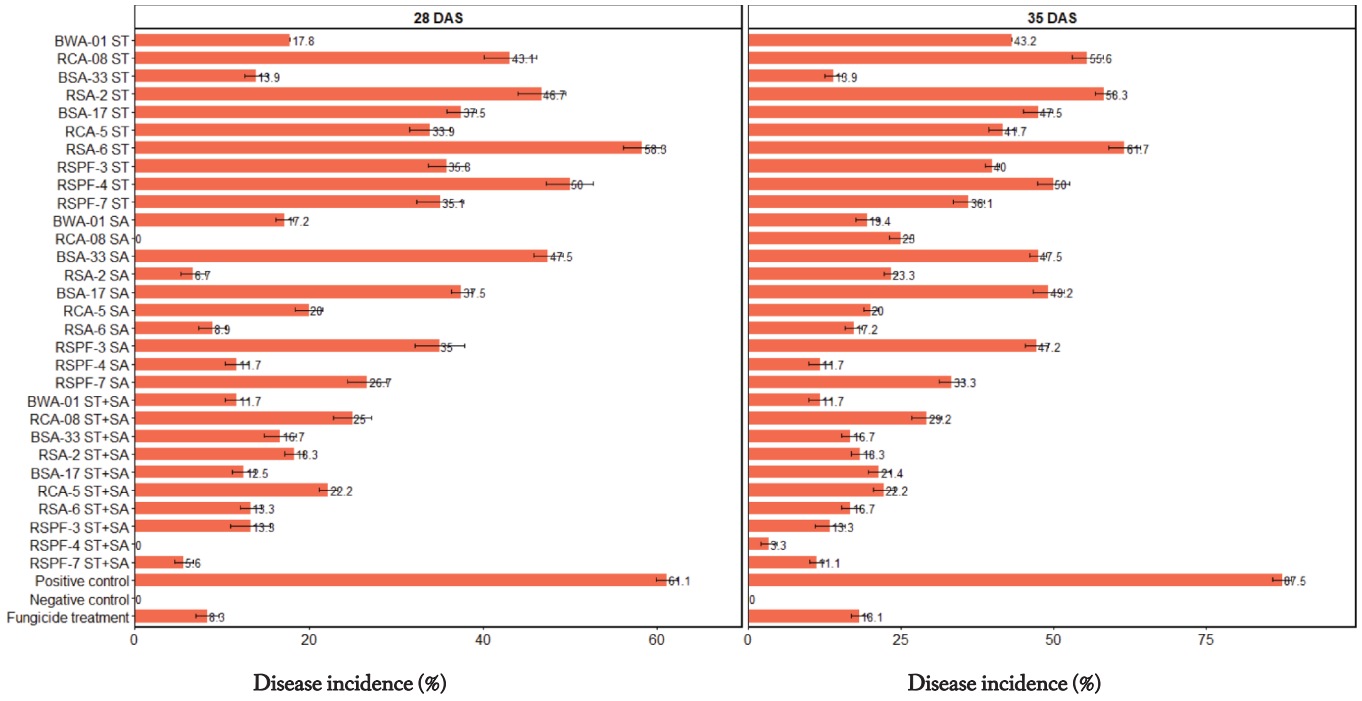


Fig. 3. Effect of potential strains against *sclerotium rolfsii* pathogen under glasshouse conditions.

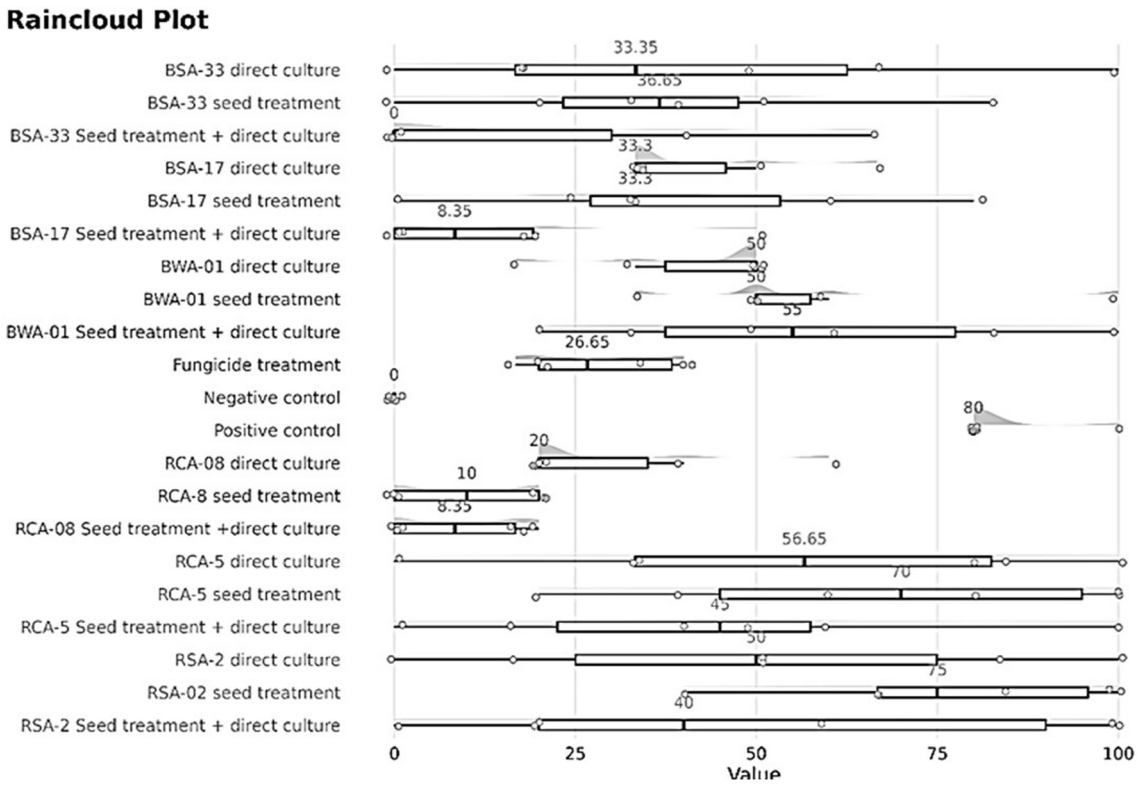
asiatica RS7. Phylogenetic trees are represented in Fig. 5.

3.6. Compatibility screening of potential isolates through cross streaking

The results revealed that all isolates were synergistic and did not show any antagonism to each other (Fig. 6). These findings indicated the potential for developing a multi-strain consortium with enhanced pathogen suppression capabilities.

3.7. Efficacy of syncom inoculants against S. rolfsii and R. bataticola under glasshouse conditions

Chickpea seedlings inoculated with only pathogen (positive control) dried completely, exhibiting 100% disease incidence (Fig. 7). Whereas chickpea seedlings treated with SynComs reduced the collar rot incidence and disease symptoms. The results were significant ($p < 0.05$) in reducing the disease incidence up to 70 – 100% in chickpea. Among the SynComs tested, SC-4 (SynCom with only actinomycetes species.) and SC-6 (SynCom with all potential isolates) resulted in less prominent symptoms and significantly reduced the disease incidence up to 100%



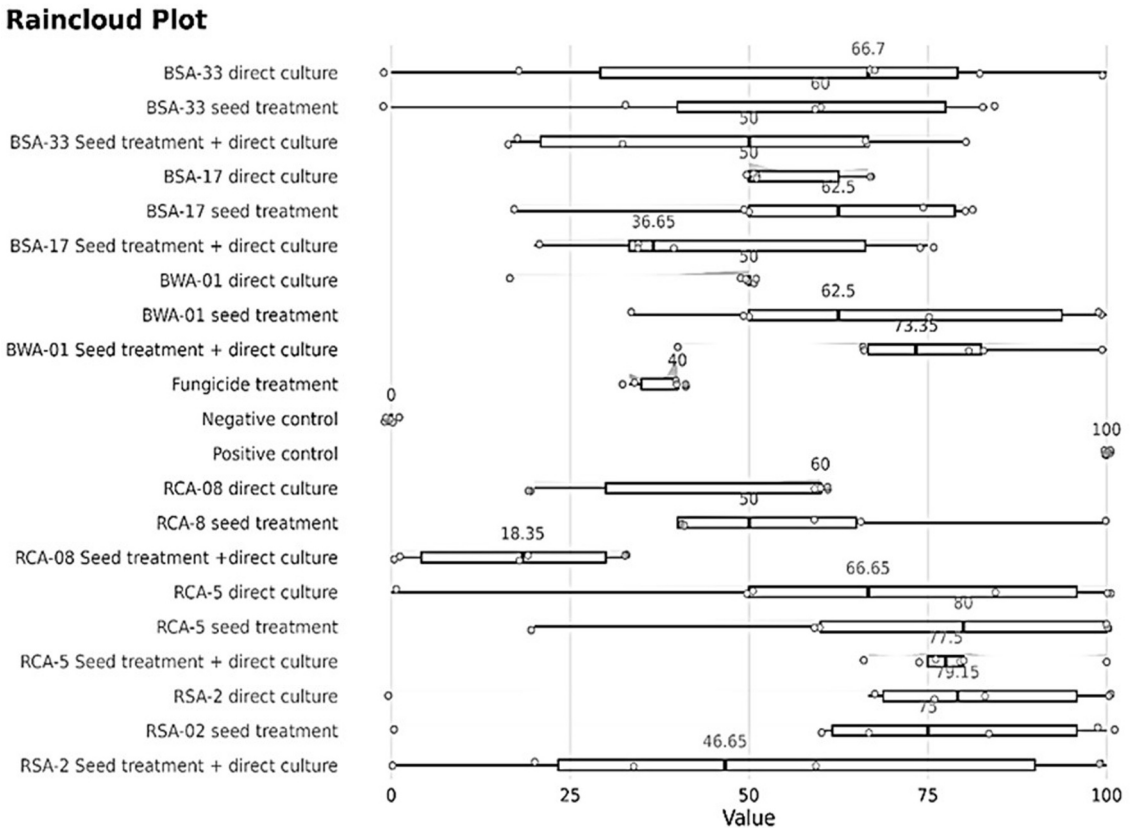


Fig. 4. Raincloud plot representing mean inhibition percentages of antagonistic isolates against dry root rot disease under glasshouse conditions at 30 and 45 DAS.

(Table 5). Remaining SynComs (SC-1, SC-2, SC-3, SC-5) showed the disease incidence of 30.6%, 21.7%, 25.0% and 27.8%. Fungicide treatment exhibited the disease incidence of 13.3%. While against *R. bataticola*, treatments with SynComs SC-1, SC-5, SC-6 recorded significantly low disease incidence. Though the roots of a few plants did exhibit lesions, but the plants did not die (disease rating 3–4). Remaining SynComs exhibited the disease incidence of 33.33% when compared to control. No disease symptoms were observed in the fungicide control. Clustered heatmap (Fig. 7) indicated clear grouping of treatments based on disease incidence. SC-2 and SC-3 were clustered

Table 4
Molecular characterization of prominent isolates.

S. No.	Isolate	16 s r RNA identification	Similarity per centage (%)	Query cover %	Accession number
1.	BSA33	*Streptomyces rochei*	100	100	PQ901976
2.	BWA01	*Nesterenkonia aethiopica*	97.51	98	PX116859
3.	BSA17	*Streptomyces sampsonii*	96.65	96	PX116863
4.	RCA5	*Streptomyces champavati*	94.91	98	PX116872
5.	RCA08	*Streptomyces harbiensis*	96.80	96	PX116880
6.	RS4	*Pseudomonas putida*	99.77	98	PX116878
7.	RS7	*Pseudomonas asiatica*	99.65	97	PX116879

together with positive control for both the diseases, reflecting higher disease incidence and less effectiveness in disease suppression. Whereas SC-4 and SC-6 exhibited lower disease incidence of collar rot indicating superior efficacy in disease suppression. SC-1, SC-5 and SC-6 demonstrated reduced disease incidence of dry root rot like chemical and negative controls.

4. Discussion

The effective management of soil-borne pathogens like *S. rolfsii* and *R. bataticola* which causes substantial crop losses under favourable conditions is crucial for sustainable agriculture. Biocontrol is one of the environmentally friendly alternatives to conventional pesticides (Borneman & Becker 2007; Singh et al. 2024). Among biocontrol agents, antagonistic bacteria are particularly promising due to their selectivity, safety and diverse modes of action (Iftikhar et al. 2020). A key advantage of these microbes is their dual functionality: they not only antagonize pathogens while also promote plant growth via mechanisms such as siderophore and ammonia production, enhancing their appeal for agricultural use (Archana et al. 2020). Herein, the study focused on disease suppression/biocontrol of chickpea soil borne pathogens under *in vivo* conditions. We demonstrated that bacterial isolates RSPF-04, BWA01, RSPF-07, BSA-33 significantly reduced disease incidence of *S. rolfsii* pathogen to $<$ 20% in glasshouse trails. This suppression is likely due to the production of secondary metabolites such as glucanases, chitinases, siderophores, lipases, proteases, etc which either inhibit pathogen or induce systemic resistance in plant. These findings align with Bidima et al. (2021), who reported that seed bacterization with four potential bacterial isolates reduces *S. rolfsii* disease incidence to 20% (treated with O4 isolate) compared to 88.9% in control at 30 days after inoculation. Similarly, Ayed et al. (2025) found that selected bacterial isolates significantly reduced *S. rolfsii* disease infection and enhanced the growth parameters in tomato plants. The ability of bacterial isolates in the present study, to reduce disease incidence indicate that they can be used in effective disease management. The potent biocontrol activity observed is likely mediated by multiple mechanisms such as competition for nutrients, production of antibiotic metabolites or induction of systemic resistance. However, to develop them into reliable biocontrol products, their precise mode of action needs to be defined that leads to this inhibition to identify prominent bacterial species.

Rhizoctonia bataticola is one of the destructive pathogens responsible for significant agricultural losses. This investigation characterized the efficacy of microbial isolates against *R. bataticola* by assessing their antagonistic activity along with their plant growth promoting properties. The *in vivo* glasshouse studies demonstrated a significant reduction in disease incidence by the individual strains. The *Streptomyces harbiensis* RCA08, recorded lowest disease incidence of 23.4%, followed by BSA-17 with 38.6% and BSA-33 with 46.7%. These findings are consistent with Diaz-Diaz- et al. (2022) who reported that disease severity significantly reduced to 21.7% to 6.4% in *Streptomyces* sp. CBQ-EA-2 treated seedlings compared to control with 70.3% disease incidence against *Macrophomina phaseolina* in *Phaseolus vulgaris*. Similarly, Singh et al. (2024) demonstrated the efficacy of biocontrol agents against *R. bataticola* in cluster bean. While *Trichoderma afroharzianum* 5F alone exhibited 42% disease control, a consortium made of *Pseudomonas fluorescens* 131B, *Bacillus subtilis* 236B, *T. afroharzianum* 5F and *Bacillus licheniformis* 223B was the most effective in reducing disease incidence. It was speculated that the potential efficacy of isolates likely stemmed from antifungal metabolites production or increased availability of nutrients. In the present study as well, the combination of different bioagents proved more effective in suppressing the disease expression.

The selected promising isolates (including actinomycetes and fluorescence *Pseudomonas*) were found to significantly enhance the plant growth promoting traits including nodule number, nodule weight, root weight, seed weight, shoot weight, harvest index under glass house conditions. There are several reports on PGP bacteria reported widely in enhancing nodulation and biomass thereby improving production and yield in crops including rice, sorghum, pearl millet, pigeon pea and chickpea (Gopalakrishnan et al. 2013; Gopalakrishnan et al. 2016; Islam et al. 2016; Sathya et al. 2016; Anusha et al. 2019; Srinivas et al. 2021; Srinivas et al. 2022). In the present study, the enhanced PGP parameters of chickpea crop was probably because of production of IAA, siderophores, hydrolytic enzymes such as protease, lipase, β-1,3-glucanase, and nutrient solubilization by these isolates. Such broad-spectrum PGP activities of selected promising isolates could be effectively used as bio stimulants for enhancing yield and productivity. Therefore, these actinomycetes are likely to be the potential candidates for the discovery of novel secondary metabolites for various biocontrol applications and growth promotion.

The identification of the selected isolates confirmed their taxonomic assignment to the well documented genera *Streptomyces* and *Nesterenkonia*, along with the species such as *Pseudomonas putida* and *Pseudomonas asiatica* which are well known beneficial microbes. *Streptomyces* spp. is known for their prolific production of diverse arsenal of antimicrobial secondary metabolites and lytic enzymes, making them formidable antagonists against a wide range of soil-borne pathogens (Bubici 2018; Kawicha et al. 2023). Similarly, *Nesterenkonia* spp. are being increasingly recognized for their biocontrol potential, demonstrating remarkable resilience to abiotic stresses and the ability to produce novel bioactive compounds with antifungal properties (Gohel et al. 2018; Wang et al. 2021). The species *Pseudomonas putida* is a well-established plant beneficial bacterium, widely studied for its superior root colonizing proficiency and multifunctional plant growth promotion through mechanisms such as siderophore production, phosphate solubilization and the induction of systemic resistance (Costa-Gutierrez et al. 2022). Whereas *Pseudomonas asiatica* has more recently been characterized as an effective biocontrol agent, capable of suppressing fungal diseases through direct antagonism and the induction of systemic resistance in host plants (Kumar et al. 2016).

The primary objective of designing a microbial consortium is to identify synergistic or additive interactions that result in superior disease suppression compared to individual components. To this end, we used the compatibility streaking technique to evaluate these microbial interactions by streaking test strains perpendicularly on agar plates and examined the intersection zones after incubation for growth inhibition (antagonism) or synergism (compatibility). Notably, all prominent isolates tested in this study were synergistic and did not show any antagonism to each other, confirming their potential in developing coherent and effective microbial consortia for enhanced disease control.

Microbial communities are known to perform diverse and valuable functions within agricultural ecosystems. Recently, microbial communities (SynComs) have emerged as a promising tool to boost crop yields by augmenting the functions of naturally occurring microbial

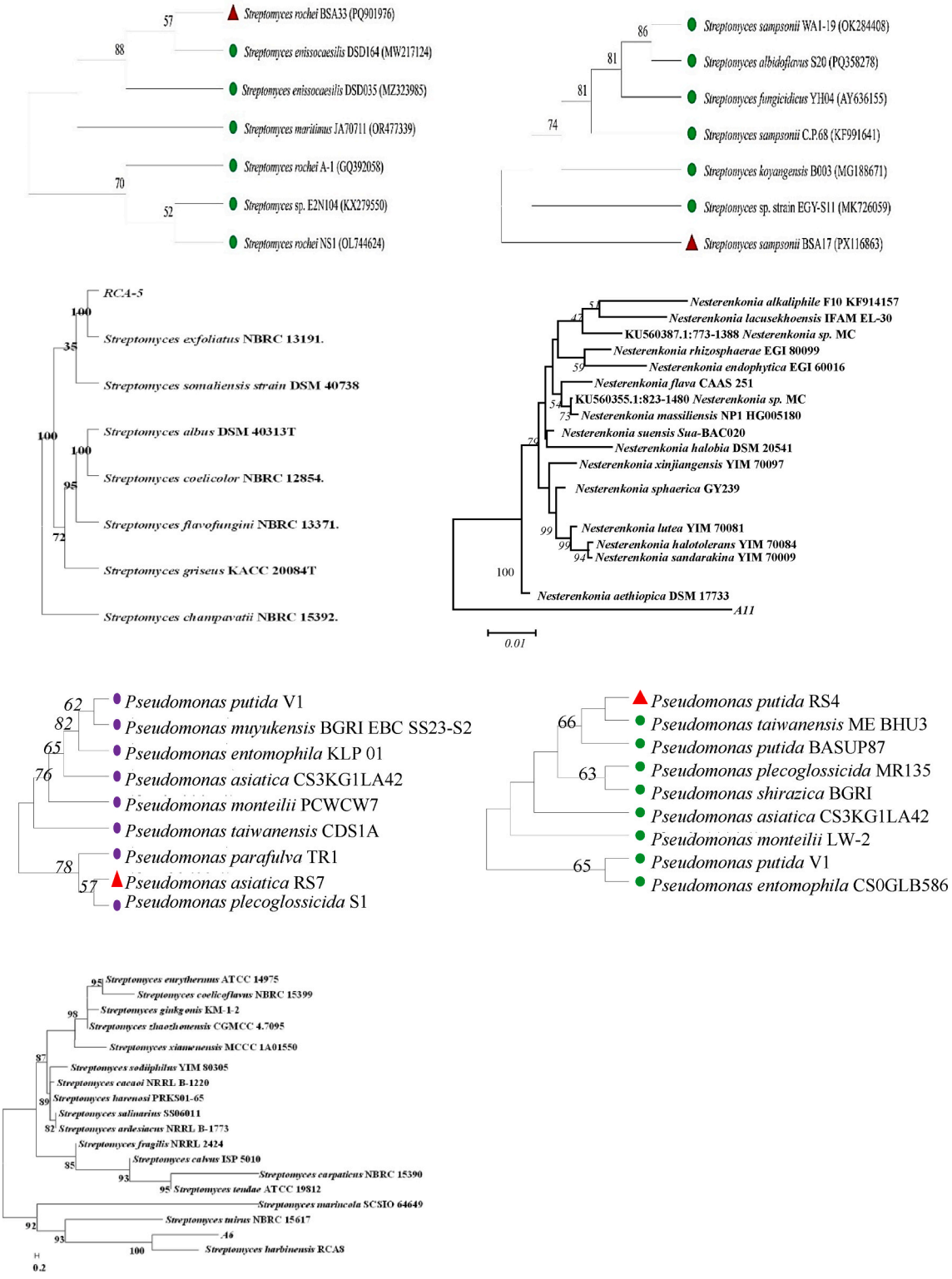


Fig. 5. Phylogenetic tree of *Streptomyces rocheii* BSA33, *Streptomyces sampsonii* BSA17, *Streptomyces champavati* RCA5, *Nesterenkonia aethiopica* BWA01, *Pseudomonas asiatica* RS7, *Pseudomonas putida* RS4, *Streptomyces harbiensis RCA8* constructed using the Neighbour-Joining (NJ) method based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch nodes, indicating the reliability of each clade.

populations (Santhanam et al. 2019; Tsolakidou et al. 2019; de Souza et al. 2020; Wang et al. 2021; Pradhan et al. 2022; Yin et al. 2022). Notably, there are no reports for SynComs designed through only actinomycetes strains but there are a very few reports on SynComs prepared with bacterial sp. along with one or two *Streptomyces* strains (Yin et al. 2022; Chen et al. 2025). To our knowledge, this is the first report that SynComs with particularly actinomycetes sp. were able to protect chickpea from collar rot and dry root rot infection. In the present investigation, 6 synthetic consortia were prepared with actinomycetes species and other bacterial isolates such as *Pseudomonas* and *Rhizobia* using cultivation-based approach. Two syncoms SC-4 (made of only actinomycetes sp.), SC-6 (made of all antagonistic isolates) resulted in

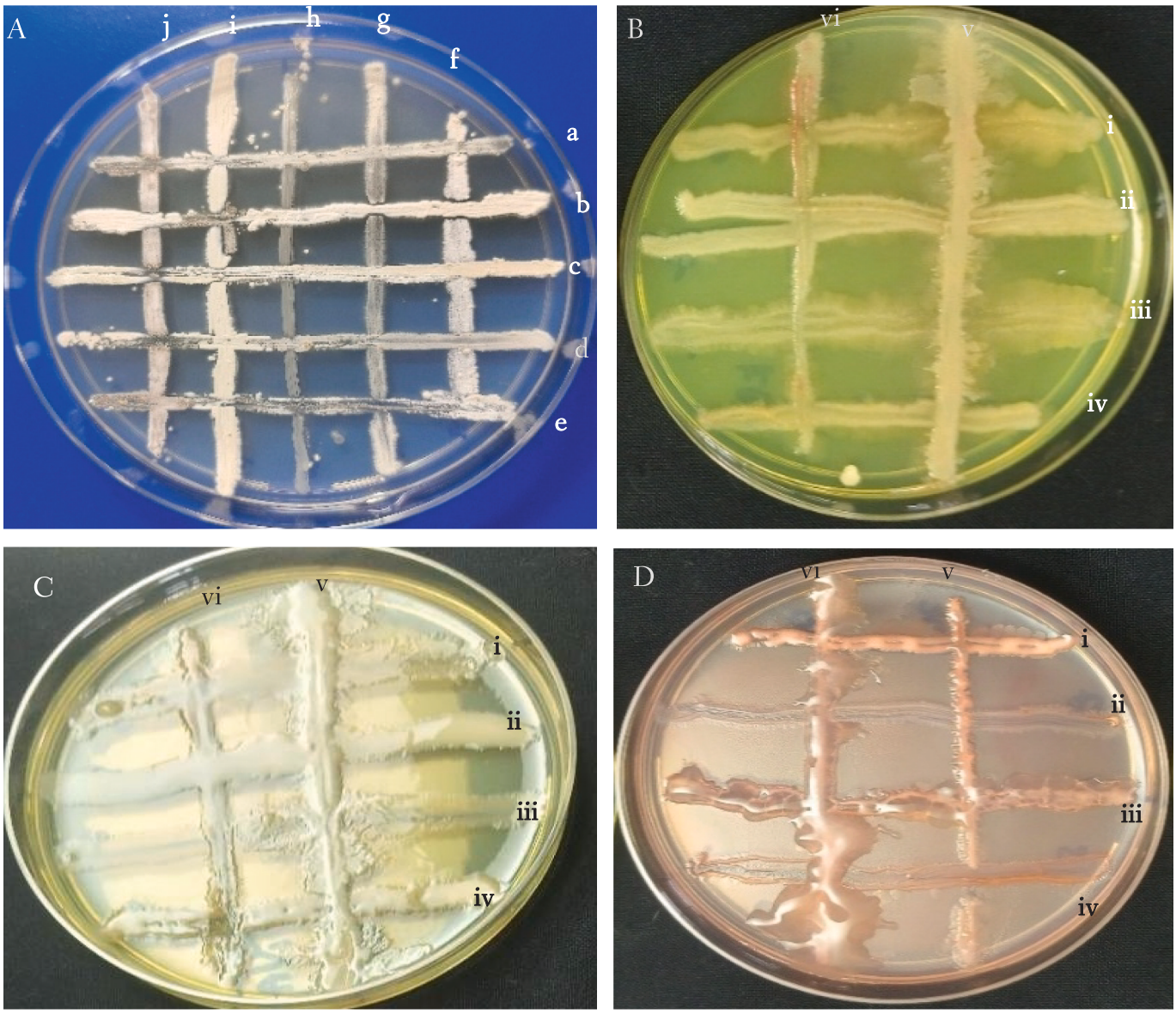


Fig. 6. Compatibility screening of selected potential isolates. A) Cross streaking of Actinomycetes isolates [BSA-33 (a), BSA-17 (b), BSA-4 (c), RSA-2 (d), RCA-5 (e), RSA-6 (f), BWA-01 (g), RCA-8 (h), BSA-20 (i), and BSA-14 (j)] on AIA media (B) Cross streaking of *Pseudomonas* [RSPF-3 (i), RSPF-4 (ii), RSPF-7 (iii) and RSPF-8 (iv)] and *Rhizobium* [IC-59 (v) and IC-76 (vi)] isolates on yeast extract mannitol agar (YEMA) media and C&D) on King's B media. Compatibility of isolates with each other is confirmed with no inhibition zone.

reduced disease incidence of *S. rolfsii*, while SC-1, SC-5, SC-6 syncoms demonstrated lesser incidence of *R. bataticola*. The results are in consistent with Tsolakidou et al. 2019 who tested the efficacy of two SynComs made of 25 different bacteria against *Fusarium* wilt disease and growth of tomato plants. Both the SynCom treatments increased the growth of tomato plants in terms of total leaf area, total number of leaves and plant height. However, SynCom-2 treated plants showed reduced disease severity up to 28.9% compared to plants treated with SynCom-1 and controls. Significant reduction in *Fusarium* wilt of banana has also been reported by Prigigallo et al. 2022 through SynComs. The results of present investigation are aligned with Yin et al. 2022 where they evaluated the efficacy of effective individual strains and ten SynComs made of 14 bacterial strains against wheat rot infection caused by *Rhizoctonia solani*. Seven out of 10 SynComs significantly reduced the infection of *R. solani* when compared to control. Overall, the results of our studies showed that SynComs reduced the collar rot symptoms and performed better than the individual/single strains. These results need to be further confirmed by screening the SynComs across multiple field trails in the target regions. In addition, there is a need to study their synergistic interactions to understand the exact biocontrol mechanism.

5. Conclusion

In the present study, isolates *Nesterenkonia aethiopica* BWA01, *Streptomyces harbiensis* RCA08, *Pseudomonas putida* RS4, *P. asciatica* RS7, *Streptomyces rocheii* BSA33, *Streptomyces sampsonii* BSA17, *Streptomyces champavati* RCA5 have been found effective with antagonistic activity against collar rot and dry root rot pathogens of chickpea along with multiple plant growth-promoting traits. The SynCom treatments achieved 100% reduction in disease incidence with milder symptoms, demonstrating their enhanced efficacy. The study provides a framework of use of PGP microbes with biocontrol properties into chickpea production systems, enhancing resilience against soil-borne pathogens while promoting crop productivity offering a scalable solution for reducing agrochemical dependence and promoting soil fertility.

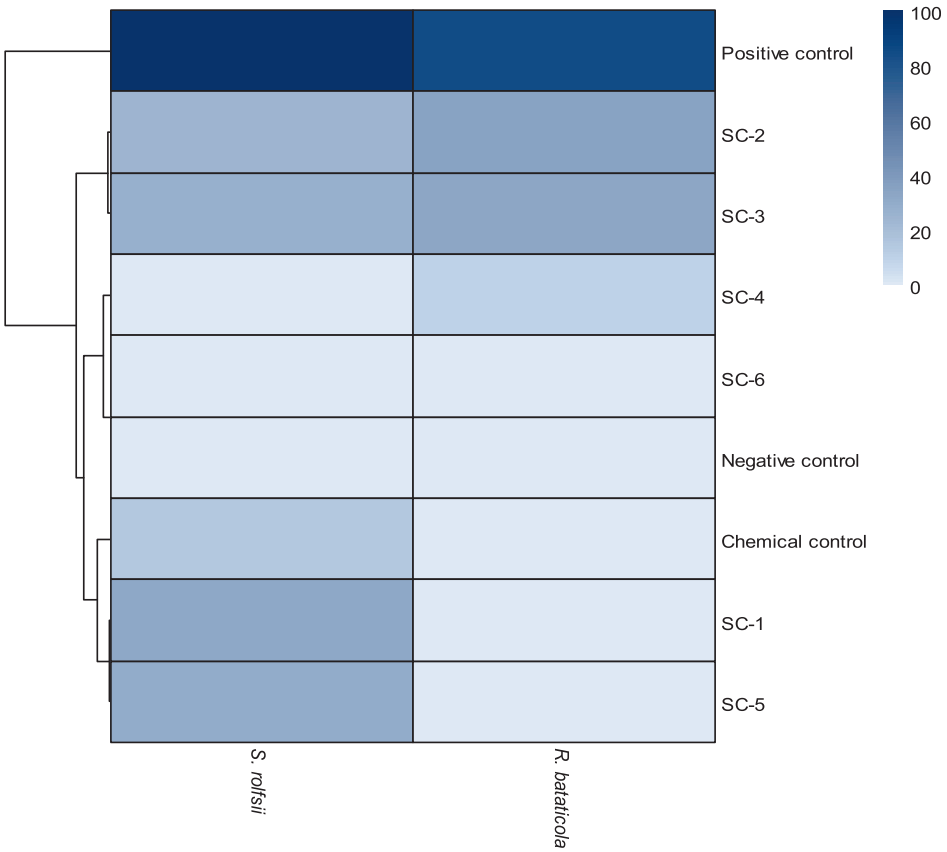


Fig. 7. Heatmaps showing the efficacy of SynCom (SC) inoculants against *S. rolfsii* and *R. bataticola* pathogens under glasshouse conditions. SC-1 to SC-6 – SynCom inoculants; positive control – only pathogen treatment; negative control – healthy uninoculated plants, and chemical control – fungicide treated plants.

Table 5
Effect of SynCom inoculants against collar rot and dry root rot pathogens under glass house conditions.

Isolate	Per cent disease incidence (PDI)	
	S. rolfsii	*R. bataticola*
SC-1	30.6 (0.59)[b]	0.0 (0.00)[b]
SC-2	21.7 (0.48)[b]	33.3 (0.52)[b]
SC-3	25.0 (0.52)[b]	33.3(0.52)[b]
SC-4	0.0 (0.00)[d]	6.7 (0.15)[b]
SC-5	27.8 (0.55)[b]	0.0 (0.00)[b]
SC-6	0.0 (0.00)[d]	0.0 (0.00)[b]
Positive control	100.0 (1.57)[a]	86.7(1.34)[a]
Negative control	0.0 (0.00)[d]	0.0 (0.00)[b]
Fungicide control	13.3 (0.31)[c]	0.0 (0.00)[b]
SE(d)	0.08	0.37
SE(m)	0.05	0.26
LSD ($P \leq 0.01$)	0.16	0.78

Values in parentheses were arcsine-transformed values. Means allotted with same letters do not differ significantly (0.05) by Tukey HSD.

6. Consent to participate

The authors declare consent to participate in this work.

7. Consent for publication

The authors declare consent to publish this work.

8. Data availability statement

All data generated or analysed during this study are included in this manuscript and can be provided on request. The nucleotide sequences referenced in table 4 have been deposited to NCBI GenBank database and are available under the accession numbers (PQ901976, PX116859-PX116879) (https://www.ncbi.nlm.nih.gov/).

CRediT authorship contribution statement

Syed Vahida Rehman: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Rajan Sharma:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Avula Vijaya Gopal:** Writing – review & editing. **Gogisetty Sravani:** Writing – review & editing, Formal analysis, Data curation. **Vadlamudi Srinivas:** Writing – review & editing, Methodology, Investigation. **Nimmala Naresh:** Methodology, Investigation. **B. Sarvani:** Writing – review & editing. **S. Gopalakrishnan:** Writing – review & editing, Methodology, Conceptualization. **Mamta Sharma:** Writing – review & editing, Supervision, Methodology, Conceptualization. **D. Ramesh:** Writing – review & editing, Software, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This research has been carried out at International Crops Research Institute for Semi-Arid Tropics (ICRISAT). The authors gratefully acknowledge the technical support and assistance provided by Legume pathology staff during the course of experiments.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215 (3), 403–410. https://doi.org/10.1016/s0022-2836(05)80360-2.

Ankati, S., Srinivas, V., Pratyusha, S., Gopalakrishnan, S., 2021. *Streptomyces* consortia-mediated plant defense against *Fusarium* wilt and plant growth-promotion in chickpea. Microb. Pathog. 157, 104961. https://doi.org/10.1016/j.micpath.2021.104961.

Anusha, B.G., Gopalakrishnan, S., Naik, M.K., Sharma, M., 2019. Evaluation of *Streptomyces spp.* and *Bacillus spp.* for biocontrol of Fusarium wilt in chickpea (*Cicer arietinum* L.). Archives of Phytopathology and Plant Protection 52 (5–6), 417–442.

Archana, T., Rajendran, L., Manoranjitham, S.K., Santhana Krishnan, V.P., Paramasivan, M., Karthikeyan, G., 2020. Culture-dependent analysis of seed bacterial endophyte, *Pseudomonas* spp. EGN 1 against the stem rot disease (*Sclerotium rolfsii* Sacc.) in groundnut. Egyptian J. Biological Pest Control 30 (1), 119. https://doi.org/10.1186/s41938-020-00317-x.

Ayed, F., Aydi Ben Abdallah, R., Ben Khedher, S., Jabnoun-Khiareddine, H., Daami-Remadi, M., 2025. Biocontrol of *Agroathelia rolfsii* associated with stem rot disease in tomato (*Solanum lycopersicum* L.) and growth promotion using compost-associated actinobacteria. Braz. J. Microbiol. 1–16. https://doi.org/10.1007/s42770-025-01647-4.

Bidima, M.G., Chtaina, N., Ezzahiri, B., El Guilli, M., 2021. Evaluation of the antagonistic potential of bacterial strains isolated from moroccan soils for the biological control of *Sclerotium rolfsii* Sacc. Int. J. Food Sci. Agric. 5 (4). https://doi.org/10.26855/ijfsa.2021.12.007.

Borneman, J., Becker, J.O., 2007. Identifying microorganisms involved in specific pathogen suppression in soil. Annu. Rev. Phytopathol. 45, 153–172. https://doi.org/10.1146/annurev.phyto.45.062806.094354.

Bubici, G., 2018. Streptomyces spp. as biocontrol agents against Fusarium species. CABI Reviews 1–15. https://doi.org/10.1079/PAVSNNR201813050.

Cao, F.Y., Yoshioka, K., Desveaux, D., 2011. The roles of ABA in plant–pathogen interactions. J. Plant Res. 124 (4), 489–499. https://doi.org/10.1007/s10265-011-0409-y.

Chandran, S, Tarafdar, A., Mahesha, H.S., Sharma, M., 2021. Temperature and soil moisture stress modulate the host defense response in chickpea during dry root rot incidence. Front. plant sci. 12, 653265. https://doi.org/10.3389/fpls.2021.653265.

Chen, C., Li, K., Lin, X., Li, W., Xu, X., 2025. Engineering natural microbial communities: harnessing synthetic communities for bioremediation. Curr. Opin. Microbiol. 87, 102652. https://doi.org/10.1016/j.mib.2025.102652.

Costa-Gutierrez, S.B., Adler, C., Espinosa-Urgel, M., de Cristóbal, R.E., 2022. *Pseudomonas putida* and its close relatives: mixing and mastering the perfect tune for plants. Appl. Microbiol. Biotechnol. 106 (9), 3351–3367. https://doi.org/10.1007/s00253-022-11881-7.

De Souza, R.S.C., Armanhi, J.S.L., Arruda, P., 2020. From microbiome to traits: designing synthetic microbial communities for improved crop resiliency. Front. Plant Sci. 11, 1179. https://doi.org/10.3389/fpls.2020.01179.

FAO, F., 2025. Available online at: http://faostat. fao. org/site/291/default. aspx. Food and Agriculture Organization.

Fisher RA (1918) The correlation between relatives on the supposition of Mendelian inheritance. Trans. Roy. Soc. Edinburgh. 52:399–433. (Reprinted with a commentary by P.A.P. Moran and C.A.B. Smith as Eugenics Laboratory Memoir XLI. Cambridge University Press, London. 1966).

Gohel, S.D., Sharma, A.K., Dangar, K.G., Thakrar, F.J., Singh, S.P., 2018. Biology and applications of halophilic and haloalkaliphilic actinobacteria. In Extremophiles (pp. 103-136). CRC Press. DOI:10.1201/9781315154695-6.

Gopalakrishnan, S., Humayun, P., Kiran, B.K., Kannan, I.G.K., Vidya, M.S., Deepthi, K., Rupela, O., 2011. Evaluation of bacteria isolated from rice rhizosphere for biological control of charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid. W J Microbiol. Biotechnol. 27 (6), 1313–1321. https://doi.org/10.1007/s11274-010-0579-0.

Gopalakrishnan, S., Srinivas, V., Sree Vidya, Rathore, A., 2013. Plant growth-promoting activities of Streptomyces spp. in sorghum and rice. SpringerPlus 2 (1), 574.

Gopalakrishnan, S., Vadlamudi, S., Samineni, S., Sameer Kumar, C., 2016. Plant growth-promotion and biofortification of chickpea and pigeonpea through inoculation of biocontrol potential bacteria, isolated from organic soils. Springerplus 5 (1), 1882.

Green, M.R., Sambrook, J., 2017. Isolation of high-molecular-weight DNA using organic solvents. Cold Spring Harb Protoc 4, 093450. https://doi.org/10.1101/pdb.prot093450.

Gupta, O.M., Sharma, M., 2015. Dry root rot of chickpea: an overview. Journal of Food Legumes 28 (4), 267–276.

Gupta, J., Singh, U.N., Akram, M., Mishra, R.K., 2021. Potential of biological control agents for the management of soil-borne pathogens in pulse crops. J. Food Legumes 34 (3), 149–165.

ICRISAT Happenings, 2019. Real-time surveillance decrypts dry root rot spread in Central India. Available online - https://www.icrisat.org/real-timesurveillance-decrypts-dry-root-rot-spread-in-central-india/ (accessed April 26, 2019).

Iftikhar, Y., Sajid, A., Shakeel, Q., Ahmad, Z., Haq, Z.U., 2020. Biological antagonism: a safe and sustainable way to manage plant diseases. In: Plant Disease Management Strategies for Sustainable Agriculture through Traditional and Modern Approaches. Springer, Cham, pp. 83–109. https://doi.org/10.1007/978-3-030-35955-3_5.

Islam, S., Akanda, A.M., Prova, A., Islam, M.T., Hossain, M.M., 2016. Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. Frontiers in Microbiology 6, 1360.

Kawicha, P., Nitayaros, J., Saman, P., Thaporn, S., Thanyasiriwat, T., Somtrakoon, K., Sangdee, K., Sangdee, A., 2023. Evaluation of soil *Streptomyces* spp. for the biological control of Fusarium wilt disease and growth promotion in tomato and banana. Plant Pathol J. 39 (1), 108. https://doi.org/10.5423/PPJ.OA.08.2022.0124.

Kumar, M., Mishra, S., Dixit, V., Kumar, M., Agarwal, L., Chauhan, P.S., Nautiyal, C.S., 2016. Synergistic effect of *Pseudomonas putida* and *bacillus amyloliquefaciens* ameliorates drought stress in chickpea (*Cicer arietinum* L.). Plant Signal Behave. 11 (1), 1071004. https://doi.org/10.1080/15592324.2015.1071004.

Kumar, K.K., Sridhar, J., Murali-Baskaran, R.K., Senthil-Nathan, S., Kaushal, P., Dara, S. K., Arthurs, S., 2019. Microbial biopesticides for insect pest management in India: current status and future prospects. J. Invertebr. Pathol. 165, 74–81. https://doi.org/10.1016/j.jip.2018.10.008.

Pande, S., Sharma, M., Avuthu, N., Telangre, R., 2012. *High Throughput Phenotyping of Chickpea Diseases: Stepwise Identification of Host Plant Resistance*. In: *Information Bulletin*, 92. International Crops Research Institute for Semi-Arid Tropics.

Pradhan, S., Tyagi, R., Sharma, S., 2022. Combating biotic stresses in plants by synthetic microbial communities: principles, applications and challenges. J. Appl. Microbiol. 133 (5), 2742–2759. https://doi.org/10.1111/jam.15799.

Prigigallo, M.I., Gómez-Lama Cabanás, C., Mercado-Blanco, J., Bubici, G., 2022. Designing a synthetic microbial community devoted to biological control: the case study of *Fusarium* wilt of banana. Front. Microbiol. 13, 967885. https://doi.org/10.3389/fmicb.2022.967885.

Rai, A., Irulappan, V., Senthil-Kumar, M., 2022. Dry root rot of chickpea: a disease favored by drought. Plant Dis. 106 (2), 346–356. https://doi.org/10.1094/PDIS-07-21-1410-FE.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4 (4), 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454.

Santhanam, R., Menezes, R.C., Grabe, V., Li, D., Baldwin, I.T., Groten, K., 2019. A suite of complementary biocontrol traits allows a native consortium of root-associated bacteria to protect their host plant from a fungal sudden-wilt disease. Mol. Ecol. 28 (5), 1154–1169. https://doi.org/10.1111/mec.15012.

Sathya, A., Vijayabharathi, R., Srinivas, V., Gopalakrishnan, S., 2016. Plant growth-promoting actinobacteria on chickpea seed mineral density: an upcoming complementary tool for sustainable biofortification strategy. *3*. Biotech 6 (2), 138.

Sharma, M., Pande, S., 2013. Unravelling effects of temperature and soil moisture stress response on development of dry root rot [*Rhizoctonia bataticola (Taub.)*] Butler in Chickpea. Am. J. Plant Sci. 4 (3), 584–589. https://doi.org/10.4236/ajps.2013.43076.

Sharma, M., Ghosh, R., Tarafdar, A., Telangre, R., 2015. An efficient method for zoospore production, infection and real-time quantification of *Phytophthora cajani* causing *Phytophthora* blight disease in pigeon pea under elevated atmospheric CO_2. BMC Plant Biol. 15 (1), 1–12. https://doi.org/10.1186/s12870-015-0470-0.

Simmons, T.B., 2020. Investigating the causes and consequences of drought-induced endophytic actinobacteria enrichment. University of California, Berkeley.

Singh, D., Geat, N., Jadon, K.S., Verma, A., Sharma, R., Rajput, L.S., Mahla, H.R., Kakani, R.K., 2024. Isolation and characterization of biocontrol microbes for development of effective microbial consortia for managing *Rhizoctonia bataticola* root rot of cluster bean under hot arid climatic conditions. Microorganisms 12 (11), 2331. https://doi.org/10.3390/microorganisms12112331.

Srinivas, V., Sravani, A., Pratyusha, A., Gopalakrishnan, S., 2021. Amazing plant growth-promoting actinobacteria from herbal vermicompost. Andhra Pradesh Journal of Agricultural Sciences 7 (2), 89–98.

Srinivas, V., Naresh, N., Pratyusha, S., Ankati, S., Govindaraj, M., Gopalakrishnan, S., 2022. Exploring plant growth-promoting *Streptomyces* spp. for yield and nutrition traits in pearl millet hybrids. Crop and Pasture Science 73 (5), 484–493.

Statista, 2023. https://www.statista.com/statistics/722203/chickpeas-production-volume-by-country-worldwide.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25 (24), 4876–4882. https://doi.org/10.1093/nar/25.24.4876.

Tripathi, G., Rawal, S.K., 1998. Simple and efficient protocol for isolation of high molecular weight DNA from *Streptomyces aureofaciens*. Biotech. Techniques. 12, 629–631. https://doi.org/10.1023/A:1008836214495.

Tsolakidou, M.D., Stringlis, I.A., Fanega-Sleziak, N., Papageorgiou, S., Tsalakou, A., Pantelides, I.S., 2019. Rhizosphere-enriched microbes as a pool to design synthetic communities for reproducible beneficial outputs. FEMS Microbiol. Ecol. 95 (10), 138. https://doi.org/10.1093/femsec/fiz138.

Tukey, J.W., 1977. Exploratory data analysis. *Reading/Addison-Wesley*. Xvi 688, 24.

Yin, C., Hagerty, C.H., Paulitz, T.C., 2022. Synthetic microbial consortia derived from rhizosphere soil protect wheat against a soilborne fungal pathogen. Front. Microbiol. 13, 908981. https://doi.org/10.3389/fmicb.2022.908981.