International Journal of Plant & Soil Science

Volume 38, Issue 11, Page 62-69, 2026; Article no.IJPSS.168059
ISSN: 2320-7035

Evaluation of a Microbial Consortium for Growth Promotion and Suppression of Sclerotial Rot of Groundnut

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2026/v38i116401

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/168059

Original Research Article

Received: 17/08/2026
Accepted: 12/10/2026
Published: 31/10/2026

Abstract

Sclerotial rot caused by *Sclerotium rolfsii* is a soil-borne disease of groundnut and is difficult to manage because of the persistent nature of the pathogen in soil. This study investigated a compatible microbial consortium comprising *Rhizobium* sp. R5, potassium-mobilising bacteria (KMB) isolate K1, *Bacillus megaterium* and *Pseudomonas fluorescens* for improving groundnut growth and reducing sclerotial rot. The four selected bacterial isolates showed no visible inhibition zone in the compatibility test and were combined in equal proportions (1:1:1:1). Their effect on seed germination and seedling growth was first evaluated by the rolled paper towel method and subsequently under pot conditions using groundnut cv. TAG-73. The consortium recorded 97.00% germination, 9.10 cm root length, 9.83 cm shoot length and a seedling vigour index of 1836.21 under rolled paper towel conditions. In pot culture, the consortium recorded 90.00% germination, 9.93 cm root length, 14.16 cm shoot length and the highest seedling vigour index of 2168.10. An incidence of sclerotial rot of 22.41% was recorded following consortium application compared with 57.93% in the untreated control, corresponding to 61.31% disease reduction relative to the control. The results indicate that the compatible four-member microbial consortium has the potential to improve early plant growth and suppress *S. rolfsii* under pot conditions.

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Cite as: *Suryawanshi, A. U., Gaikwad, M. S., Chandurkar, R. S., Shelke, P. V., Atram, P. C., Ingle, Y. V., & Gahukar, S. J. (2026). Evaluation of a Microbial Consortium for Growth Promotion and Suppression of Sclerotial Rot of Groundnut. International Journal of Plant & Soil Science, 38(11), 62–69. https://doi.org/10.9734/ijpss/2026/v38i116401*

Keywords: Arachis hypogaea; Sclerotium rolfsii; microbial consortium; seed treatment; disease management; Rhizobium; pseudomonas fluorescens; Bacillus megaterium.

1. Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed and food crop cultivated in many parts of the world. In India, its production is affected by several biotic and abiotic stresses. Among these diseases, stem or sclerotial rot caused by *Sclerotium rolfsii* is important because the pathogen survives in soil through persistent sclerotia (Vamshi *et al*., 2022) and can infect plants near the soil surface. The pathogen produces abundant white mycelium and sclerotia around infected tissues, resulting in rotting, wilting and plant death (Punja, 1985; Guclu *et al*., 2020; Hawaladar *et al*., 2022). The management of *S. rolfsii* is difficult because the pathogen has a wide host range and survives in soil for long periods (Chandurkar *et al*., 2025). Chemical fungicides can provide disease suppression, but their repeated use may have limitations related to cost, environmental effects and the development of reduced sensitivity in pathogen populations (Zatale *et al*., 2026). Therefore, biological approaches using beneficial microorganisms have received increasing attention as components of sustainable disease management (Mehan *et al*., 1994). Divyasree *et al.* (2024) reported differences in the *in vitro* inhibitory activity of bacterial and fungal bioagents against *S. rolfsii* associated with groundnut stem rot.

Plant growth-promoting microorganisms can support plant establishment through nutrient mobilisation, phytohormone production and interactions with the rhizosphere (Compant et al., 2019). Combining beneficial microorganisms may provide complementary functions, but compatibility must be assessed because interactions among consortium members can be antagonistic or neutral (Negi et al., 2024; Nunes et al., 2024). Syed et al. (2020) reported beneficial effects of a plant growth-promoting rhizobacterial consortium on groundnut seed germination and root growth, while Kampa et al. (2025) described the potential of compatible microbial consortia for managing groundnut collar rot and chickpea wilt. Seed biopriming with actinobacteria has also been associated with improved peanut seedling vigour (Suryawanshi and Krishnaraj, 2020). Bacillus megaterium has been studied for nutrient mobilisation and plant growth-promoting traits (Amalraj et al., 2012), and Madhan et al. (2025) reported improved groundnut growth following treatments involving selected plant-associated bacteria and signalling molecules.

Microbial combinations have also been investigated for the management of *S. rolfsii*. Meena et al. (2024) reported that combinations involving Trichoderma harzianum and *P. fluorescens* reduced stem rot incidence and improved groundnut performance; the best treatment in that study recorded 7.40% disease incidence and 69.37% disease control under the tested conditions. Nidheesh (2024) also examined microbial bioconsortia for groundnut stem rot. Chang et al. (2025) reported that seed treatment with Bacillus siamensis YB-1632 promoted peanut growth and suppressed *S. rolfsii* blight under greenhouse conditions. Balamurugan et al. (2026) reported *in vitro* inhibition of *S. rolfsii* mycelial growth by Trichoderma viride culture filtrates.

However, the performance of the specific combination of *Rhizobium* sp. R5, KMB isolate K1, *B. megaterium* and *P. fluorescens* in relation to early groundnut growth and sclerotial rot incidence under pot conditions has not been established in the studies cited above.

The present study evaluated a four-member consortium comprising *Rhizobium* sp. R5, KMB isolate K1, *B. megaterium* and *P. fluorescens* for its effects on seed germination, seedling growth and sclerotial rot incidence in groundnut under laboratory and pot conditions.

2. Materials and Methods

The investigation was conducted during 2025–26 at the Department of Plant Pathology, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India. A pot experiment was conducted using groundnut cultivar TAG-73 at the Oilseeds Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola.

2.1 Isolation and Identification of *Sclerotium rolfsii*

Diseased groundnut stems showing characteristic symptoms of sclerotial rot were collected and brought to the laboratory. Small pieces of infected stem tissue were cut from the advancing margin of the diseased area. The pieces were surface sterilised with 0.1% mercuric chloride for 2 min, washed and transferred aseptically onto potato dextrose agar (PDA). Potato dextrose agar (PDA) medium was prepared using 200 g of peeled potatoes, 20 g of dextrose, and 20 g of agar, with distilled water added to make up a final volume of 1000 mL. The plates were incubated at 27 ± 2°C. The developing fungal growth was transferred to fresh PDA plates and purified. A seven-day-old cultures were used for further studies. The fungus produced white, cottony mycelial growth followed by the formation of sclerotia that changed from white to brown and later became dark brown. Microscopic observations were recorded at 40× magnification using a compound microscope. The characteristics observed were compared with the standard descriptions of *S. rolfsii* (Punja, 1985).

2.2 Pathogenicity Test

The pathogen *S. rolfsii* was mass-multiplied on sorghum grains. One kilogram of sorghum grains was mixed with 250 mL water and transferred into 2000 mL conical flasks. The flasks were autoclaved at 121.6°C and at approximately 1.04 kg cm^{-2} pressure for 15 min and the process was repeated on the following day. After cooling, the grains were inoculated with mycelial plugs from a pure culture of *S. rolfsii* and incubated at 27 ± 2°C for 15 days following Shokes *et al*. (1996). The flasks were shaken on alternate days during incubation. The colonised sorghum grains were subsequently used to prepare pathogen-infested soil (Bekriwala *et al*., 2016). Pathogenicity was then confirmed using groundnut plants grown in sick soil, following Awasthi *et al*. (2010). Symptoms developed

approximately seven days after sowing. White fungal growth and sclerotia were observed around the basal portion of diseased plants, followed by wilting and easy uprooting. The pathogen was re-isolated from diseased tissues to confirm the causal organism.

2.3 Preparation of Soil

Sandy loam soil with a neutral reaction was used. The soil was sieved and treated with formalin using formalin-treated cotton balls, then left for 15 days to allow residual formalin to dissipate. Subsequently, 2 kg of treated soil was placed in each pot. The formalin concentration and amount applied should be specified in the final version to make the procedure reproducible.

2.4 Selection and Preparation of Microbial Isolates

Four bacterial isolates, namely *Rhizobium* sp. R5, KMB isolate K1, *Bacillus megaterium* and *Pseudomonas fluorescens*, were selected on the basis of their desirable plant-beneficial traits and compatibility and were subsequently employed for the development of a bacterial consortium. The *Rhizobium* isolate R5 showed a positive reaction for IAA production, as indicated by the development of a pink to reddish colour following the addition of Salkowski reagent, as described by Bric *et al.* (1991). KMB isolate K1 showed the highest potassium-mobilising activity among the tested isolates. The selected bacterial isolates were maintained on suitable nutrient media for further experimentation. *Bacillus megaterium* isolate was assessed qualitatively using Pikovskaya's agar medium by spot inoculation. Also, the antagonistic effect of *Pseudomonas fluorescens* against *Sclerotium rolfsii* was studied under *in vitro* conditions using the dual culture technique and the isolates selected respectively.

2.5 Compatibility Test

Compatibility among the four selected bacterial isolates was assessed by the disc diffusion method. The test organisms were grown on nutrient agar and observed for the development of inhibition zones. No visible inhibition zone was observed among the four selected isolates, indicating compatibility under the conditions of the test. The absence of an inhibition zone was considered evidence of compatibility (Chamkhi *et al*., 2023).

2.6 Preparation of Microbial Consortium

The selected compatible isolates, *Rhizobium* sp. R5, KMB K1, *Bacillus megaterium* and *Pseudomonas fluorescens*, were individually grown in 100 mL of sterile nutrient broth in 250 mL conical flasks, with each flask inoculated with 5 mL of an actively growing culture. The flasks were incubated on a rotary shaker at 100 rpm to obtain a uniform population of approximately 10^8 CFU mL^{-1}. The population was confirmed by serial dilution and plate count. To prepare the consortium, equal volumes (25 mL) of the four culture suspensions were mixed in a sterile conical flask (1:1:1:1 v/v/v/v) to make a total of 100 mL. Glycerol was added at 0.5 mL L^{-1} as a stabiliser and carboxymethyl cellulose (CMC) at 0.01 g L^{-1} (0.001% w/v) as an adhesive/carrier, and the mixture was shaken to ensure thorough mixing (Shahzad et al., 2017).

2.7 Seed Treatment and Experimental Treatments

Groundnut seeds were treated with individual microbial cultures, the microbial consortium and a fungicide before sowing. The consortium treatment consisted of *Rhizobium* sp., KMB, *B. megaterium* and *P. fluorescens* in equal proportions. The treatments used in the pot culture experiment are detailed in Table 1.

Table 1. Details of microbial treatments used for evaluation

Treatments	Treatment details
T_1	*Rhizobium* @10ml/kg seed
T_2	KMB coded @10ml/kg seed
T_3	*Bacillus megaterium* @10ml/kg seed
T_4	*Pseudomonas fluorescens* @10ml/kg seed
T_5	Consortium $T_1+T_2+T_3+T_4$
T_6	Mancozeb 75% WP @2.5 g kg^{-1} seed
T_7	Untreated Control

2.8 Germination

The efficacy of individual bacterial isolates, the microbial consortium and the fungicide was evaluated using healthy seeds of groundnut cv. TAG-73. The seeds were divided into seven lots and each lot was assigned the respective treatment. *Rhizobium* sp., potassium-mobilising bacteria (KMB), *Bacillus megaterium*, *Pseudomonas fluorescens*, microbial consortium and Mancozeb 75% WP. The seventh lot was maintained as an untreated control. The treated seed lots were stored at room temperature in the laboratory for a few days before evaluation. Seed germination and seedling growth were assessed by the rolled paper towel method, as described by Priya et al. (2019). Germination was recorded after seedling emergence and the percentage was calculated according to the International Rules for Seed Testing (International Seed Testing Association, 2003).

2.9 Root and Shoot Length

Root and shoot lengths were recorded from representative seedlings and expressed in centimetres on day 14. The mean values were used for comparison among treatments (Abdul-Baki and Anderson, 1973).

2.10 Seedling Vigour Index

Seedling vigour index was calculated using the following formula as suggested by Abdul-Baki and Anderson (1973).

Seedling vigour index = Germination percentage × (Root length + Shoot length)

2.11 Pot culture Experiment

The treated seeds were sown in pots containing sterilised soil. Pathogen-infested soil was prepared using the *S. rolfsii*-colonised sorghum grain inoculum. Three replications were maintained for each treatment. Observations on seed germination, root length, shoot length, seedling vigour index and disease incidence were recorded.

2.12 Disease Incidence

Disease incidence was recorded by counting the number of diseased plants in each treatment and expressed as a percentage of the total number of plants observed.

2.13 Disease Reduction over Control

Disease reduction relative to the untreated control was calculated as:

$$\text{Disease reduction (\%)} = \frac{[\text{ Incidence in control} - \text{Incidence in treatment})}{\text{Incidence in control}} \times 100$$

3. Results and Discussion

3.1 Effect of Microbial Treatments on Seed Germination and Seedling Growth by Paper Towel Method

The effect of individual microbial treatments and the consortium on germination and early seedling growth is presented in Table 2.

The consortium treatment (T5) recorded the highest germination of 97.00%, followed by *B. megaterium* with 95.33%. The untreated control recorded 88.00% germination. The consortium also recorded the highest shoot length (9.83 cm) and root length (9.10 cm). The corresponding values in the untreated control were 3.50 and 3.33 cm, respectively. The highest seedling vigour index of 1836.21 was recorded in the consortium treatment, whereas the untreated control recorded 601.04. Thus, the consortium produced an approximately 205.51% higher SVI than the untreated control. The improvement in early seedling growth may be related to the different beneficial characteristics of the microorganisms included in the consortium. Plant-associated beneficial bacteria can influence plant development through nutrient mobilisation, phytohormone production and other plant growth-promoting activities.

The present findings are consistent with the general observations of Syed *et al*. (2020), who reported beneficial effects of PGPR consortium treatment on groundnut seed germination and root growth.

Table 2. Effect of microbial treatments on seed germination and seedling growth of groundnut under rolled paper towel conditions

Sr. No.	Treatment details	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling vigour index (SVI)
T_1	*Rhizobium* @10ml/kg seed	92.33 (74.07)*	4.33	5.17	877.135
T_2	KMB coded @10ml/kg seed	92.33 (74.07)	4.57	6.20	994.39
T_3	*Bacillus megaterium* @10ml/kg seed	95.33 (77.54)	7.50	7.80	1458.54
T_4	*Pseudomonas fluorescens* @10ml/kg seed	90.00 (71.57)	4.67	5.67	930.6
T_5	Consortium T1+T2+T3+T4	97.00 (80.12)	9.83	9.10	1836.21
T_6	Mancozeb 75% WP @2.5 g kg^{-1} seed	91.67 (73.31)	3.17	5.50	794.77
T_7	Untreated Control	88.00 (71.89)	3.50	3.33	601.04
	SE (m) ±	1.10	0.62	0.80	100.4
	CD (P = 0.01)	4.62	2.63	3.36	422.67

**Figures in parentheses are angular-transformed values*

Gupta *et al*. (2020) also reported improved germination and seedling vigour in peanut following treatment with a plant growth-promoting *Pseudomonas* isolate. The present experiment differed in bacterial composition and experimental conditions, but both studies demonstrate the potential of beneficial bacterial inoculation to improve early groundnut establishment.

3.2 Effect of Microbial Treatments on Plant Growth and Disease Incidence under Pot Conditions

The performance of different microbial treatments on seed germination, seedling growth and disease incidence of groundnut under pot culture conditions was assessed, and the results are presented in Table 3.

The highest germination of 90.00 per cent was recorded in the microbial consortium treatment (T5), followed by *Rhizobium* sp. (T1) and KMB (T2), each recording 86.67 per cent. *P. fluorescens* (T4) and Mancozeb (T6) recorded 83.33 per cent germination. *B. megaterium* (T3) recorded 73.33 per cent, whereas the untreated control (T7) recorded the lowest germination of 63.33 per cent. The higher germination percentage observed with the consortium indicates a favourable response of groundnut seeds to combined microbial inoculation. The consortium contained microorganisms with different plant-beneficial properties, which may have contributed to improved early seedling establishment.

Similar results indicating improvement in germination and seedling growth following the application of beneficial microbial combinations have been reported by Trivedi *et al*. (2020) and Kampa *et al*. (2025). The microbial consortium recorded the highest root length of 9.93 cm, followed by KMB (6.16 cm), *Rhizobium* sp. (7.10 cm), *P. fluorescens* (7.00 cm), Mancozeb (7.90 cm) and *B. megaterium* (5.60 cm). The untreated control recorded the shortest root length of 4.30 cm. A similar response was observed for shoot growth. The consortium recorded the highest shoot length of 14.16 cm. *Rhizobium* sp., *B. megaterium*, *P. fluorescens*, Mancozeb and KMB recorded shoot lengths of 11.90, 11.06, 10.10, 8.53 and 9.83 cm, respectively, while the untreated control recorded 7.20 cm. Improved root and shoot development following microbial treatment may be associated with the plant growth-promoting properties of the selected microorganisms. Beneficial rhizobacteria can contribute to nutrient mobilisation and production of plant growth-promoting substances, thereby supporting early plant development.

Table 3. Effect of microbial consortium on growth promotion and disease suppression under pot culture conditions

Tr. No.	Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling vigour index (SVI)	Disease incidence (%)	Disease reduction over control (%)
T_1	*Rhizobium* @10ml/kg seed	86.67 (68.85)*	7.10	11.90	1646.73	31.02 (33.71)	46.45
T_2	KMB coded @10ml/kg seed	86.67 (68.85)	6.16	9.83	1332.44	32.41 (34.54)	44.05
T_3	*Bacillus megaterium* @10ml/kg seed	73.33 (59.70)	5.60	11.06	1221.67	32.80 (34.75)	43.37
T_4	*Pseudomonas fluorescens* @10ml/kg seed	83.33 (66.14)	7.00	10.10	1424.94	31.94 (34.34)	44.86
T_5	Consortia $T_1+T_2+T_3+T_4$	90.00 (74.90)	9.93	14.16	2168.10	22.41 (28.23)	61.31
T_6	Mancozeb 75% WP @2.5 g kg^{-1} seed	83.33 (66.14)	7.90	8.53	1423.98	33.33 (31.12)	53.65
T_7	Untreated Control	63.33 (52.77)	4.30	7.20	728.29	57.93 (49.61)	0
	SE (m) ±	4.88	0.64	0.74	118.33	2.70	
	CD (P=0.01)	20.54	2.71	3.14	498.19	11.36	

**Figures in parentheses are angular transformed values*

Taurian *et al*. (2010) reported plant growth-promoting characteristics among peanut-associated bacteria, while Gupta *et al*. (2020) observed improved germination, root development and seedling vigour following treatment with a plant growth-promoting *Pseudomonas* isolate. In addition, the highest seedling vigour index of 2168.10 was recorded in the microbial consortium treatment. This was followed by *Rhizobium* sp. (1646.73), *P. fluorescens* (1424.94), KMB (1332.44), Mancozeb (1423.98) and *B. megaterium* (1221.67). The untreated control recorded the lowest seedling vigour index of 728.29. The higher seedling vigour index in the consortium treatment was associated with its higher germination percentage, root length and shoot length. The combined microbial treatment therefore promoted overall seedling development under the experimental conditions. Similar beneficial effects of microbial combinations on seedling establishment have been reported by Trivedi *et al*. (2020).

In the present study, all the microbial treatments reduced disease incidence compared with the untreated control. The lowest disease incidence of 22.41 per cent was recorded in the consortium treatment (T5), whereas the untreated control recorded the highest disease incidence of 57.93 per cent. Among the individual microbial treatments, KMB recorded 32.41 per cent disease incidence, followed by *P. fluorescens* (31.94%), *Rhizobium* sp. (31.02%) and *B. megaterium* (32.80%). The fungicide treatment with mancozeb recorded a disease incidence of 33.33%. The reduction in disease incidence following microbial treatment indicates the potential contribution of beneficial microorganisms to the suppression of *S. rolfsii*. The response of the consortium may be associated with the combined action of microorganisms having different plant growth-promoting and antagonistic properties. Kampa *et al*. (2025) similarly demonstrated that compatible microbial consortia could improve plant growth and reduce *S. rolfsii*-associated collar rot of groundnut.

3.3 Disease Reduction over Control

The microbial consortium recorded the highest disease reduction over the untreated control, with a 61.31 per cent reduction. KMB, *Rhizobium* sp., *P. fluorescens*, Mancozeb and *B. megaterium* recorded disease reductions of 44.05, 46.45, 44.86, 53.65 and 43.37 per

cent, respectively. The comparatively greater reduction observed with the four-member consortium suggests that combining compatible microorganisms provided a broader beneficial effect than their individual application under the tested pot conditions. The use of microbial combinations containing organisms with complementary plant growth-promoting and biocontrol properties has also been reported to improve disease suppression in other crop systems (Trivedi *et al*., 2020).

3.4 Overall Performance of Microbial Consortium

The combined microbial treatment recorded 90.00 per cent germination, 9.93 cm root length, 14.16 cm shoot length and a seedling vigour index of 2168.10. Disease incidence was 22.41 per cent, corresponding to 61.31 per cent disease reduction over the untreated control. The untreated control recorded 63.33 per cent germination, 4.30 cm root length, 7.20 cm shoot length, a seedling vigour index of 728.29 and 57.93 per cent disease incidence. The complete results are presented in Table 3.

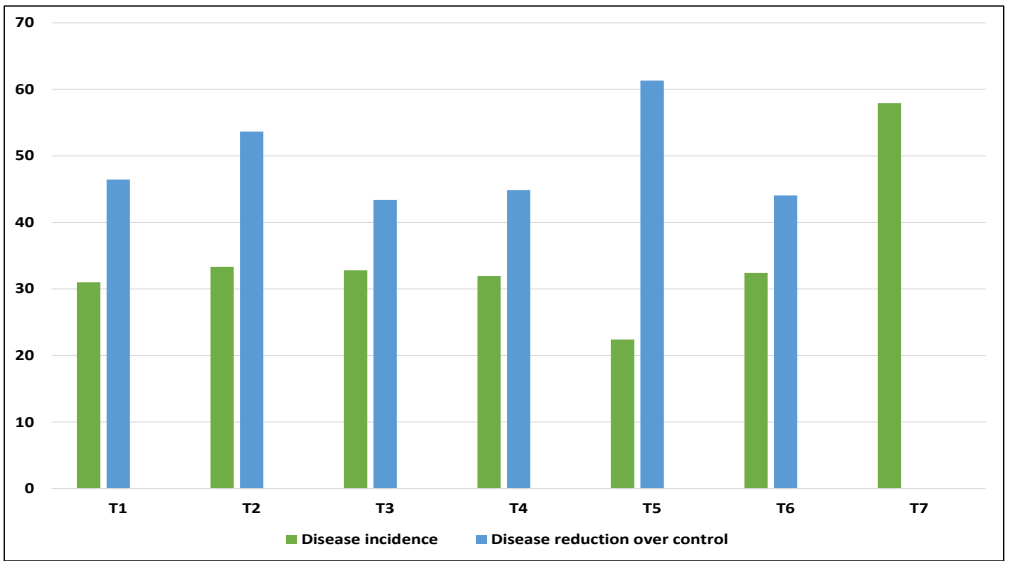

Fig. 1. Effect of microbial treatments on incidence of sclerotial rot of groundnut under pot conditions

The disease incidence data are presented graphically in Fig. 1. The highest disease incidence was recorded in the untreated control (57.93%), whereas the lowest incidence was observed in the microbial consortium treatment (22.41%).

4. Conclusion

A four-strain microbial mixture containing *Rhizobium* sp. R5, KMB K1, *Bacillus megaterium* and *Pseudomonas fluorescens* showed favourable effects on groundnut seed germination, seedling growth and suppression of sclerotial rot caused by *Sclerotium rolfsii*. The consortium recorded 97.00% germination and an SVI of 1836.21 under rolled paper towel conditions. Under pot conditions, it recorded 90.00% germination, 9.93 cm root length, 14.16 cm shoot length and an SVI of 2168.10. Disease incidence was 22.41% compared with 57.93% in the untreated control, representing a 61.31% disease reduction relative to the control. These findings underscore the promise of this compatible biocontrol consortium for plant growth promotion and disease management in groundnut, although field validation and mechanistic studies are essential before widespread adoption.

5. Limitations

The study was conducted under laboratory and pot conditions using a single groundnut cultivar (TAG-73), with three replications per treatment in the pot experiment. The use of sterilised soil and artificial pathogen inoculation may limit the generalisability of the findings to natural field conditions. Microbial compatibility was assessed through the absence of visible inhibition zones, without establishing the persistence or interactions of consortium members in soil. Furthermore, the mechanisms underlying disease suppression were not investigated. Future studies should evaluate the consortium across different cultivars, soil conditions and field environments to establish its consistency and practical applicability.

Acknowledgement

The authors are thankful to the Department of Plant Pathology, Dr. PDKV, Akola, and the Oilseed Research Unit, Dr. PDKV, Akola, for providing the facilities and support required for conducting the present investigation.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference

formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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