RESEARCH ARTICLE

Harnessing Biocontrol Agents to Combat Stem Rot Disease in Peanut Cultivation: A Sustainable Approach for Enhanced Yield and Soil Health

Riddhi H. Rajyaguru[1], Jignasha T Thumar[2,] Thirumalaisamy P P[3], Neelam Nathani[4], Chandrashekar Mootapally[4], Manju Shri[4*]

DOI: 10.18811/ijpen.v11i03.15

Abstract

Peanut (*Arachis hypogaea* L.) stands as a crucial oilseed crop in the tropical and subtropical regions of India, esteemed for its high nutritional value, comprising proteins, carbohydrates, vitamins, and minerals. Yet, its yield faces significant threats from diverse biotic and abiotic stresses prevalent in today's agricultural landscape. Among the biotic stressors, soil- and seed-borne diseases such as collar rot/crown rot/seedling blight (*Aspergillus niger*), stem rot (*Sclerotium rolfsii*), and afla rot (*A. flavus*) emerge as major concerns. However, excessive fungicide application, while traditionally employed to combat these diseases, poses risks of soil pollution and disruption of microbial diversity. In response, the exploration of biocontrol agents presents a more sustainable alternative. In the present investigation, nine bacterial isolates were scrutinized for their efficacy as biocontrols against the *S. rolfsii* fungus. Notably, *Bacillus amyloliquefaciens* (N6) and *B. subtilis* (N9) emerged as promising candidates, exhibiting substantial reductions in pathogen growth and disease symptoms, while also promoting enhanced plant growth. This study heralds a novel avenue for mitigating stem rot disease and bolstering peanut production through the application of biocontrols, heralding a promising stride towards sustainable agriculture practices.

Highlights

This investigation highlighted the efficacy of *Bacillus amyloliquefaciens* (N6) and *B. subtilis* (N9) in suppressing *Sclerofium rolfsii* growth and reducing disease symptoms in peanuts.

These bacterial isolates not only offer biocontrol against stem rot disease but also promote plant growth, potentially enhancing peanut yield and advancing sustainable agricultural practices.

The study's findings underscore the potential of utilizing beneficial bacterial isolates as an eco-friendly alternative to traditional fungicides in peanut cultivation.

By harnessing the biocontrol capabilities of *Bacillus* species, farmers can effectively manage soil- and seed-borne diseases while minimizing environmental impact.

This research paves the way for sustainable disease management strategies, fostering resilience in peanut production systems.

Keywords: Peanut (*Arachis hypogaea* L.), Soil and seed-borne diseases, Biocontrol, *Sclerotium rolfsii*, Sustainable agriculture practices.

International Journal of Plant and Environment (2025); **ISSN:** 2454-1117 (Print), 2455-202X (Online)

Introduction

Peanut (*Arachis hypogaea* L.) is an economically important oilseed crop grown in tropical and subtropical regions of India. A high level of proteins, carbohydrates, vitamins, and minerals makes peanuts nutritionally rich and healthy. Apart from that, all the plant parts from seed to leaf can be utilized as feed, making it profitable for growers. Peanut cultivation may be affected by environmental factors like temperature, amount of rainfall and its distribution. World production of peanuts was approximately 47 m metric tons in 2020, with China being the world's largest producer (https://www.peanutsusa.com/about-apc/the-peanut-industry.html). In recent years, India has planted groundnuts over nearly 4.96 million ha, yielding approximately 10.2 million metric tons in 2023 (https://www.theworldranking.com/statistics/912)

The standard productivity of peanuts in India has been low (2239 kg ha^{-1}, TE 2022-23) due to the attack of many biotic and abiotic stresses. Among the biotic stresses, soil- and seed-borne diseases like collar rot/crown rot/seedling blight (*Aspergillus niger*), stem rot (*Sclerotium rolfsii*), and aflatoxin (*Aspergillus flavus*) are categorized as major diseases (Jadon *et al*., 2015). *A. flavus* is also known to cause post-harvest contamination

[1]ICAR-Indian Institute of Groundnut Research, Junagadh, Gujarat, India.

[2]Department of Microbiology, Government Science College, Gandhinagar, India.

[3]ICAR-National Bureau of Plant Genetic Resources Regional Station, Thrissur, India.

[4]School of Applied Sciences and Technology: Gujarat Technological University, Chandkheda, Ahmedabad, India.

***Corresponding author:** Manju Shri, School of Applied Sciences and Technology: Gujarat Technological University, Chandkheda, Ahmedabad, India, Email: manjubiochem20@gmail.com

How to cite this article: Rajyaguru, R.H., Thumar J.T., Thirumalaisamy P.P., Nathani, N., Mootapally, C., Shri, M. (2025). Harnessing Biocontrol Agents to Combat Stem Rot Disease in Peanut Cultivation: A Sustainable Approach for Enhanced Yield and Soil Health. International Journal of Plant and Environment. 11(3), 575-582.

Submitted: 30/09/2024 **Accepted:** 10/07/2025 **Published:** 30/09/2025

of peanuts with aflatoxins during storage (Pandey *et al.,* 2019), which limits the export potential of peanuts. In India, major peanut consignments were redundant during export because of aflatoxin contamination. Annual yield losses in peanuts were up to 25% due to stem rot and up to 40% due to collar rot (Sanjel *et al*.,2024).

Incidence and severity of diseases may vary from season to season. Disease management programs mostly rely on pesticides, and hardly ever growers choose alternatives such as cultural, physical, biological methods, etc. Bio-agents inhibit or suppress the growth of soil-borne pathogens by one or more mechanisms. Their mode(s) of action against disease-causing pathogen(s) include, but are not limited to, competition for space or nutrients with pathogens, production of various bio-active substances with antibiotic and cell wall-degrading activity, and induction of systemic resistance in the host. The bio-agents with antifungal properties include several species of fungi, *Trichoderma viride*, *T. harzianum* and *T. asperellum*; actinomycetes, *Streptomyces* sp.; arbuscular mycorrhizal fungi, *Glomus fasciculatum*; and bacteria, *Bacillus subtilis*, *Pseudomonas fluorescens* and *P. aeruginosa* (Abd-Allah and El-Didamony, 2007; Adhilakshmi *et al.,* 2014; Biswas and Sen, 2000; Doley and Jite, 2013; Ganesan *et al.,* 2007; Krishna Kishore *et al.,* 2005; Latha *et al.,* 2011; Manjula *et al.,* 2004; Poosapati *et al.,* 2014). Many species of *Trichoderma* are commercially available and widely used by growers to control soil-borne diseases of peanut (Saravanakumar *et al.,* 2017).

Bacterial bio-agents like *Bacillus* sp. are harmless to non-target species, including humans, and hence are effectively used in post-harvest treatments of edible plants. (Ye *et al.* (2021) used *Bacillus amyloliquefaciens* for post-harvest loquat fruit storage and reported this bacterium as non-toxic. In an *in-vitro* study, *B. amyloliquifacience* gave 67% inhibition of soil-borne fungi, *S. minor* in peanut (Luduena *et al.,*2012). Similarly, *B. amyloliquefaciens* out of 37 endophytic strains of bacteria recorded significantly higher reduction (84.5%) in bacterial wilt incidence over the control in peanut (Wang and Liang, 2014). According to Buensanteai *et al.* (2008), *B. amyloliquefaciens* strain KPS 46 has reduced the severity of bacterial pustules up to 50% in soybean and increased the root and shoot length. Alvarez *et al.* (2012) have identified cyclic compounds from the lipopeptide family from two strains of *B. amyloliquefaciens* (ARP23 and MEP218), which alter the mycelia morphology and sclerotial germination of *S. sclerotiorum*. Similarly, Dunlap *et al.* (2013) identified five non-ribosomal peptide synthetase clusters encoding three lipopeptides and an antibiotic dipeptide from *B. amyloliquefaciens*. Volatile and non-volatile compounds produced by *B. amyloliquefaciens* have antifungal properties (Yuan *et al.,* 2015), whereas compounds released from *B. subtilis* improved the seed germination, nodulation, and plant growth in peanut (Turner and Backman, 1991). Microbial antagonists are widely used for the biocontrol of fungal plant diseases. Microbial interactions between antagonistic microorganisms and plant pathogens are widespread in nature (Fridlender *et al.,* 1993). Biological control of plant pathogens can be highly effective, especially with hyper hyperparasitizing potentials of antagonists on pathogenic fungi. Microorganisms either induce a defense response or produce bio-chemicals which destroy pathogens before penetration, and act as a bio-control agent to prevent disease.

In this study, nine rhizospheric bacterial strains (lab ID N1-N9 with respective GenBank accessions from KT600022-KT600030) inhibited radial colony growth of *S. rolfsii* ranging from 15.8 to 56.6% as described by Rajyaguru *et al.* (2017). Bacterial strains N1, N4 and N8 were identified as *Bacillus sp.*; N2, N3 and N9 were *B. subtilis*; and the remaining (N5, N6 and N7) were *B. amyloliquefaciens*. Bacterial strains, N6 and N5, were known to produce salicylic acid (17.7 and 3.9 µg/mL, respectively) and induce disease resistance in peanut (Rajyaguru *et al.,* 2017). These bacterial strains were further evaluated for their biocontrol activity against major soil-borne pathogens of peanut, *S. rolfsii*, *A. flavus* and *A. niger*. Compatibility between bacterial strain and seed dressing pesticides was also evaluated, along with the application method.

Materials and Methods

Fungal pathogens and bacterial strains

One virulent isolate of *S. rolfsii* (SR-8), collected from peanut fields of ICAR-Directorate of Groundnut Research, Junagadh, India, was separately maintained as pure cultures on potato dextrose agar (PDA) plates at 28°C. Sclerotia of *S. rolfsii* (SR-8) were also separately collected from PDA plates. *S. rolfsii* isolates (SR-1 to SR-45) collected from different peanut growing regions of India were also maintained as pure cultures on PDA. Bacterial strains (N1 to N9) collected from the peanut rhizosphere were maintained as pure cultures on PDA plates at 28°C.

Co-culturing assay to identify biocontrol potential of bacterial strains

Bacterial strain was grown along with the pathogen in a petri plate (9 cm dia.) containing PDA following the co-culturing technique (Oldenburg *et al.,* 2005). A bacterial colony collected on a sterilized loop was streaked on media and at equidistance; a mycelial disk (1 cm dia.) bored from the actively growing region of the pathogen was placed. In another experiment, bacterial strains were co-cultured with individual sclerotia of *S. rolfsii*. For each bacterial strain and pathogen combination, the setup was replicated four times, along with pathogen-only petri plates and incubated at 28°C. The radial colony growth (cm) of pathogens, *S. rolfsii*, was measured at 24 h intervals until the colony growth in the control covered the entire petri plate. Colony growth inhibition (%) was calculated using the below given formula, and later data were subjected to analysis of variance at $P<0.05$ significance using DSAASTAT software (Onobri, 2007).

$$\text{Colony growth inhibition (\%)} = \frac{\text{Radial colony growth of pathogen in control plate}}{\text{Radial colony growth of pathogen in co-culture Plate}} \times 100$$

Growth promotion assays

Growth promotion assays were set up on *Arachis hypogea cv.* GG-20 with nine bacterial strains treatment, along with one untreated control. About 30 seeds were taken in a flask; either bacterial inoculum or distilled water was added (250 mL) and placed on a rotary shaker for 30 min at 100 rpm. Soaked seeds were collected, shade dried on a paper towel and planted in plastic pots (12 cm dia.) containing planting mixture

Table 1: Colony growth inhibition (%) in pathogens

Bacterial Strain	*Colony growth inhibition (%) in the pathogen*			
	S. rolfsii		*A. flavus*	*A. niger*
	Sclerotia	*Mycelial disk*	*Mycelial disk*	*Mycelial disk*
N1	73.1 (58.8) d	22.5 (28.2) e	30.0 (33.5) b	29.3 (33.0) b
N2	90.7 (72.3) c	41.7 (40.2) c	42.2 (40.8) b	37.4 (38.0) b
N3	68.0 (55.6) e	15.8 (23.4) f	11.5 (20.2) c	16.7 (24.3) c
N4	75.1 (60.1) d	18.6 (25.5) ef	5.2 (12.5) de	4.8 (12.9) d
N5	59.3 (50.4) f	18.8 (25.6) ef	9.6 (18.5) cd	7.0 (15.4) d
N6	100.0 (90.0) a	56.6 (48.8) a	55.6 (48.5) a	53.1 (47.1) a
N7	19.5 (26.2) g	20.4 (26.8) e	7.0 (13.6) cde	1.1 (6.9) e
N8	94.4 (76.4) b	51.0 (45.6) b	32.4 (35.0) b	55.6 (48.5) a
N9	64.9 (53.7) e	29.7 (33.0) d	1.5 (7.5) e	5.6 (12.7) d
Control	1.0 (5.7) h	1.0 (5.7) g	1.5 (7.5) e	1.0 (5.7) e
Sem±	0.8	1.0	2.5	2.4
CD (P=0.05)	2.4	3.0	7.5	7.1
CV (%)	2.6	5.8	16.9	15.4

Means of four replications. Values in the parentheses are *$\sqrt{X}$ and **Arcsine (X+0.5) transformed. Values followed by similar alphabet are not significant at CD (p <0.05) by using DMRT.

(equal proportions of sterilized sand and peat moss). Plants were maintained in glasshouse conditions till they reached physiological maturity. Thirty plants in each treatment were grouped into five replications, with each replication comprising six plants. A week after planting, germination (%) was recorded by counting the total number of germinated seeds out of the total number planted. Two weeks after planting, pathogen *S. rolfsii* inoculum in the form of infected sorghum grains (2 g) was placed at the base of the plant. Six weeks after planting, soil plant analytical development (SPAD) chlorophyll meter reading (SCMR) was recorded on fully opened apex leaf using Minolta SPAD chlorophyll meter (Minolta Corp., Ramsey, NJ, USA. Plant wilting (%) was noted in eight-week-old plants by counting the number of plants showing wilting symptoms. After plant harvest, plant height (cm), root length (cm), root nodules (number), root volume (cm^3) and fresh weight (g) were measured from each plant. Data were subjected to analysis of variance at p <0.05 significance using DSAASTAT software. When analysis of variance showed significant treatment effects, Duncan's multiple range test (DMRT) was applied to make comparisons among the means.

In-vitro sclerotial germination inhibition assay

Sterilized soil (180 g) was mixed with 20 mL suspension ($9X10^8$ cfu/mL) of bacteria N1-9 strains. Amended soil was transferred to four sets of petri plates at 50 g/plate. About 50 sclerotia of *S. rolfsii* isolate SR-8 were spread on top of the soil and given a drenching spray of sucrose-methanol solution (dissolve 3 g of sucrose and 1.3 mL of methanol in distilled water and make up the volume to 100 mL). A control (no bacteria) set was placed for comparisons. Treated and control sets were incubated at 28°C until all the sclerotia in the control set germinated. Sclerotia germination (%) was recorded by counting the total number of germinated sclerotia out of the total sclerotia. Data were subjected to analysis of variance at p <0.05 significance using DSAASTAT software. When analysis of variance showed significant treatment effects, Duncan's multiple range test (DMRT) was applied to make comparisons among the means.

Seed-dressing pesticide tolerance spectrum of bacterial strains

The tolerances of bacterial strains (N1 to N9) to various commercial seed-dressing pesticides were studied as follows. In 100 mL PDA media was amended with any of the following pesticide *viz.*, carbendazim 50 WP (798 mg), pyraclostrobin + metiram 60 WG (1620 mg), trifloxystrobin + tebuconazole 75 WG (132 mg), difenoconazole 250 EC (0.10 mL), tebuconazole 2 DS (450 mg), tebuconazole 25.9 EC (0.15 mL), ergon 44.3 SC (0.02 mL), imidacloprid 70 WS (0.2 mg), quinalphos 25 EC (0.60 mL), and chlorpyriphos 20 EC (1.20 mL). Amended PDA media was later poured into petri plates (9 cm dia.) and 10 µL of bacterial suspension ($9X10^8$ cfu/mL) was placed. Six replications were incubated for each bacterial strain at 28°C for 48 hours. The PDA plates without pesticide served as controls. After 24 h of incubation, growth in bacterial colony was noted and categorically grouped in to good (+++), moderate (++), slight (+) and no (-).

Evaluation of bacterial strain N6 against 45 isolates of *S. rolfsii*

Forty-four isolates of *S. rolfsii* collected from different peanut growing regions of India, along with the SR-8 isolate, were maintained as pure cultures on PDA media. These isolates were co-cultured with bacterial strain N6 (*B. amyloliquefaciens*), which

was very promising in earlier studies. About 1-cm diameter mycelial disk of the pathogen was placed on PDA at equidistance from the bacterial streak, as described above. These co-cultured plates were replicated four times, along with one set of control (pathogen only) and incubated at 28 °C. The radial colony growth (cm) of the pathogen was recorded at 24 h intervals until the pathogen growth in the control covers the entire petri plate. Colony growth inhibition (%) was calculated as described above and the data were subjected to ANOVA.

Evaluation of the bacteria delivery methods of peanut

Methods for effective delivery of bacteria, N6, to *Arachis hypogea cv.* GG-20 plants were evaluated in this study. Methods evaluated include seed treatment with bacterial suspension and a talc-based formulation of bacteria. In the former method, 200 g of seeds were soaked in 250 mL of bacterial suspension ($9X10^8$ CFU/mL) for 30 min. Soaked seeds were shade-dried and planted in concrete blocks (10X3X3 cubic ft) filled with equal quantities of sand and peat moss. Seeds were planted in three rows with a row spacing of 30 cm and plant-to-plant spacing of 10 cm. In the latter method, *i.e.*, talc-based formulation of bacteria, seeds (200 g) were soaked in distilled water (250 mL) for 30 min, shade dried and planted in concrete blocks as described above. Talc-based formulation was prepared following Krishnamurthy and Gnanamanickam (1998), where 500 mL of bacterial suspension ($9X10^8$ CFU/mL) was aseptically mixed with one kg of talcum powder to obtain the formulation strength of $3X10^8$ CFU/g. This talc-based formulation was stored in polythene bags until usage. Five weeks after planting, a talc-based formulation (10 g) was placed at the base of the plant. A control was kept by soaking seeds only in distilled water for 30 min, shade-dried dried and planted in a concrete block. Each row in the concrete block was considered as a single-row replication. Pathogen, *S. rolfsii* (SR-8), was challenge inoculated to 7-week-old plants using sorghum grain inoculum (2 g/plant). A week after planting, observations were recorded on seed germination (%) while plant mortality (%) was noted in 9-week-old plants. Data were subjected to analysis of variance at $P<0.05$ significance using DSAASTAT software. When analysis of variance showed significant treatment effects, Duncan's multiple range test (DMRT) was applied to make comparisons among the means.

Data analysis

The inhibition of colony growth, seed germination, sclerotia germination, and shoot infection was respectively calculated using the following formulas:

Colony growth inhibition (%) = (Colony diameter of control - Colony diameter of treatment) / (Colony diameter of control) × 100;
Seed germination (%) = (No. of seeds germinated/total no. of seeds) × 100;
Sclerotia germination (%) = (No. of sclerotia germinated/total no. of sclerotia) × 100;
Shoot or stem infection (%) = (No. of stems infected in control - No. of stems infected in treatment) × 100

Data were analyzed using the two-way analysis of variance (ANOVA), and means were compared by the test of least significant difference (LSD) at $P=0.05$ using DSAASTAT software (Onobri, 2007).

Results

Effect of bio-control isolates on the growth of *S. rolfsii*

To check the bio-control potential of the isolates, they were co-cultured with *S. rolfsii*. The radial colony growth (cm) of pathogens was calculated and further, the colony growth inhibition via isolates was calculated.

The results of the dual culturing of bio-control isolate with the most potent isolate of *S. rolfsii* were mentioned in Fig 1 and Table1 where, the lowest radial colony growth (4.0 cm) and the highest colony growth inhibition (56.6%) were recorded with isolate, N6 which was found to be statistically superior over the control (9.0 cm and 1.0%) and at par with N8 (4.5 cm and 51.0%) and followed by N2 (5.3 cm and 41.7%). Isolate N6 has the maximum potential to decrease the pathogenic effect of *S. rolfsii, A. flavus* with 56.6%, 55.6% CGI, respectively. However, isolate N8 showed the maximum CGI against (55.6%) *A. niger,* followed by N6 (53.6%). These results indicate that the isolates have the potential to decrease the pathogenic effect of *S. rolfsii, A. flavus* and *A. niger* by reducing their growth.

The effect of organic compounds (volatile and non-volatile) produced by the bio-control isolates on the growth of the mycelial disk of the pathogen, S. rolfsii, was described in detail in Fig. 2. The volatiles produced by the isolate, N6 were found to be statistically superior over the rest of the isolates and the control (1.0% and 9.0 cm). N6 isolate was found to cause the highest colony growth inhibition of 88.4% and radial colony growth of 1.1 cm, and was closely followed by N8 (80.6% and 1.8 cm) and N2 (77.5% and 2.1 cm). However, the non-volatiles of the same isolates have not produced similar effect where, N8 recorded highest colony growth inhibition of 16.6% and radial colony growth of 7.6 cm and was statistically at par with N5 (16.4% and 7.6 cm), N6 (14.7% and 7.8 cm), N7 (14.0% and 7.8 cm), N2 (13.8% and 7.9cm), N9 (12.3% and 8.0 cm) and N1 (12.1% and 8.0).

The results of the effect of bio-control isolates on the colony growth of *S. rolfsii* grown on potato dextrose broth media were provided in Fig 4, where the lowest radial colony growth (1.0 cm) was recorded with N6 which was found to be statistically at par with N8 (1.5 cm), N2 (1.8 cm) and N1 (3.4 cm). Similarly, the highest

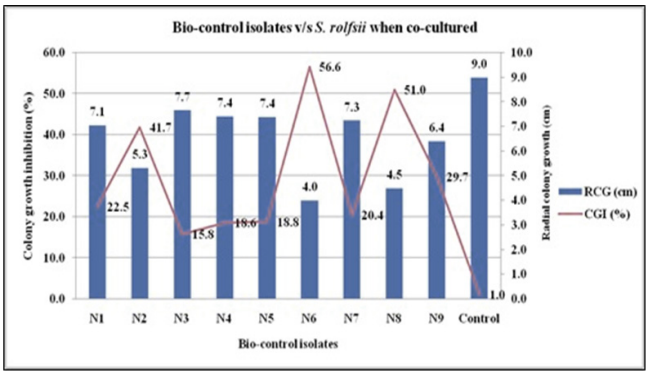

Fig. 1: Effect of bio-control isolates on mycelia growth of *S. rolfsii* in dual culture

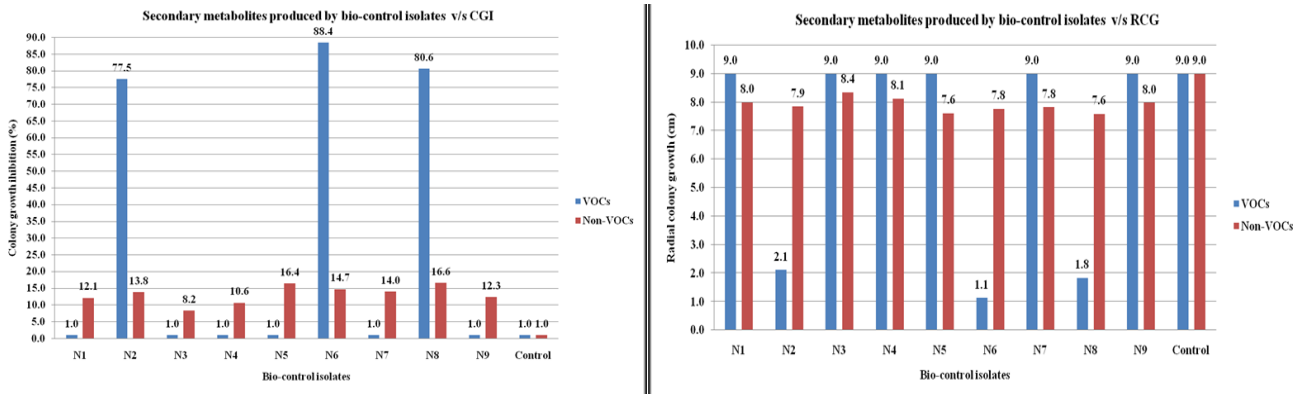

Fig. 2: Inhibitory effect of secondary metabolites produced by bio-control isolates on the growth of *S. rolfsii*. A) Effect on the colony growth inhibition (%) of *S. rolfsii.* B) Effect on radial colony growth (cm) on *S. rolfsii*

colony growth inhibition of 100.0% was recorded in isolate N6 and was found to be statistically superior to control (1.0%) and the rest of the treatments. The germination of sclerotia on sterile soil was observed and found that the sclerotia treated with all bio-control isolates caused a reduction in the germination when compared to the control (86.8%). However, the isolate, N6, was found to be statistically superior over the rest of the isolates, which recorded sclerotial germination as low as 6.2% and was followed by isolates N8 and N2 with germination of 10.8 and 17.5%, respectively.

Effect of bio-control isolates on groundnut seed germination

The observations on the germination seeds treated with bio-control isolates 4 days after pathogen inoculation as given in Fig. 5.

The highest seed germination (96.7%) was recorded with isolate N6 and was found to be statistically superior to the control (36.7%) and the rest of the isolates. This was closely followed by isolate, N8 (83.3%), which was on par with isolate, N2 (73.3%).

Effect of bio-control isolates on disease progression in groundnut

Among the nine isolates tested for the suppression of plant wilting *in-vitro* under glass house condition after 144 hours of inoculation, revealed that the isolates N6 (10.0%) and N8 (13.3%) were found to be statistically superior to the control (100%) and the remaining isolates (Fig. 6). These were immediately followed by an isolate, N2, with plant wilting of 75.0%. Similar, the trend of plant wilting suppression by the best isolates, N6, N8, and N2, was observed at 48, 72, 96, and 120 hours of inoculation.

Stem infection (%) and plant height (cm) were noted in the bio-control isolates treated seeds grown in test tubes maintained at artificial tissue culture conditions 10 days after the inoculation of the pathogen (Fig. 6).

The lowest stem infection of 8.3% was observed with isolate, N6 and was statistically at par with isolate, N8 (10.7%), and followed by isolate, N2 (22.3%). Similarly, the isolates, N6 and N8, recorded the highest plant height of 11.1 and 10.2 cm, respectively, followed by N2 (9.1 cm).

Plant growth-promoting effects of bio-control isolates in groundnut

The effect of bio-control isolates on the groundnut plant growth parameters, plant dry weight (g), plant height (cm), root length (cm), root volume (cm^3), secondary roots (no.) and root nodules (no.) were mentioned in the Fig 7.

All the parameters were found to be highest with isolate, N6 *i.e.*, 16.5 g, 48.0 cm, 17.0 cm, 0.5 cm^3, 97.3 and 64.0, respectively and was statistically superior over other isolates and control. The next best isolates are N8 (11.2 g, 42.3 cm, 14.7 cm, 0.4 cm^3, 70.0 and 44.0, respectively) and N2 (8.5 g, 39.7 cm, 14.2 cm, 0.3 cm^3, 60.0 and 35.0, respectively).

Discussion

Plant pathogens pose a significant threat to agricultural and forestry production, causing diseases that have both economic and environmental implications. Current trends in crop protection focus on reducing dependence on traditional pesticides and mandating the adoption of integrated pest

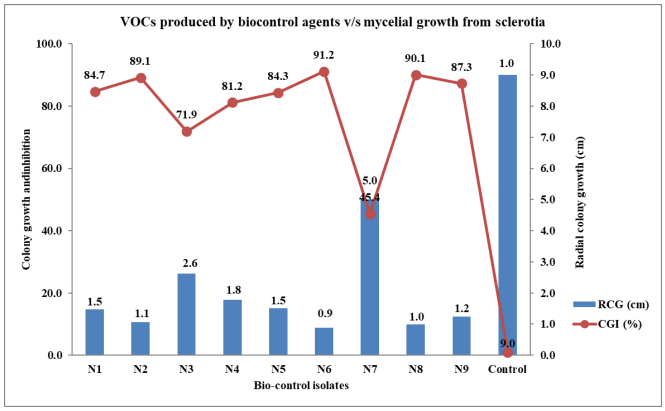

Fig. 3: Effect of VOCs produced by bio-control agents on colony growth inhibition (%) of *S. rolfsii* depicts the confirmatory results on the effect of volatile organic compounds on the sclerotia of *S. rolfsii* the isolate, N6 with a radial colony growth of 0.9 cm and colony growth inhibition of 91.2% was found to be superior over the control (9.0 cm and 1.0%) and at par with the isolates, N8 (1.0 cm and 90.1%) and N2 (1.1 cm and 89.1%).

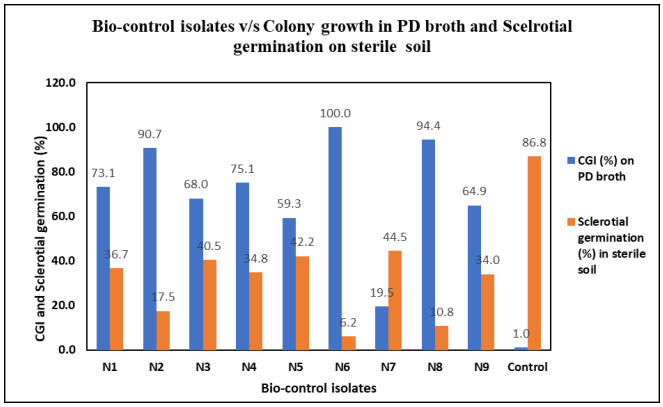

Fig 4: Effect of bio-control isolates on colony growth inhibition and scelrotial germination on sterile soil and on Potato dextrose agar media

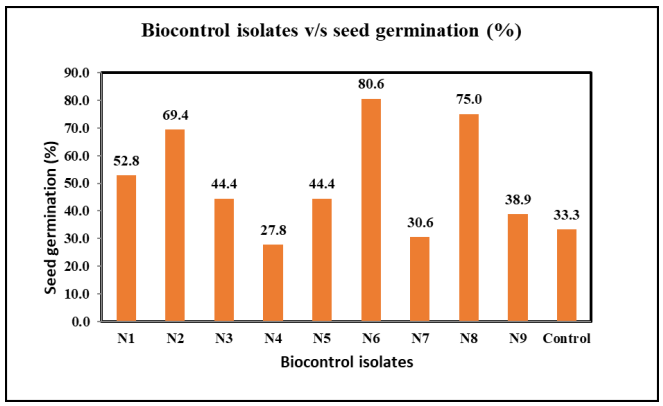

Fig. 5: Effect of bio-control isolates on seed germination of *Arachis hypogea*

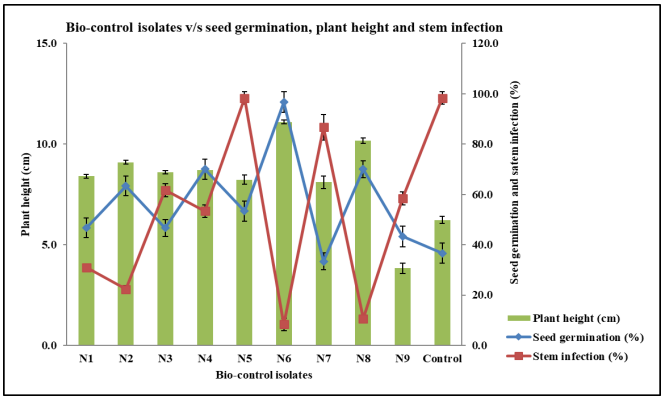

Fig. 6: Effect of bio-control isolates on stem infection by *S. rolfsii* and plant height of *Arachis hypogea* in greenhouse conditions

management (IPM) principles, as outlined in the regulations of various countries (https://eur-lex.europa.eu/Lex UriServ/LexUri Serv.do ?ur i=OJ:L:2009: 309:0071:0086:en:PDF, Lamichhane *et al.,* 2016). Consequently, there is a growing interest in effective and sustainable alternatives to conventional pesticides. Biological control emerges as a promising solution, leading to the development of a diverse range of microbial biocontrol agents (BCA) over the past decades to manage fungal and bacterial diseases.

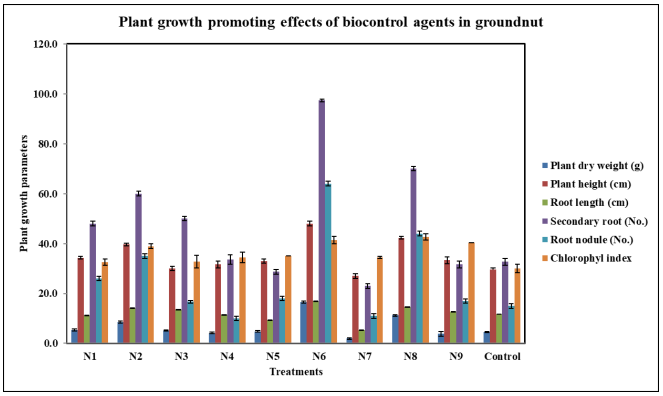

Fig 7: Effect of bio-control isolates on different plant growth parameters such as number of root nodule, number of secondary roots, root length, plant height and plant dry weight of Arachis hypogea

Recent studies have demonstrated the positive impact of bioinoculants on peanut growth. For instance, *Bacillus siamensis* YB-1632 was found to enhance seedling vigor while suppressing *Sclerotium rolfsii*, the causal agent of stem rot (Chang *et al*., 2025). Another study reported that co-inoculation with *Bradyrhizobium* and *Trichoderma harzianum* significantly improved biomass and chlorophyll content in peanuts under greenhouse conditions (Neelipally *et al*., 2020). Similarly, the application of arbuscular mycorrhizal fungi along with phosphorine biofertilizers improved yield and protein content in field-grown peanuts (Ci *et al*., 2023).

Bacteria from the genera *Pseudomonas*, *Bacillus*, and *Streptomyces* are among the most extensively researched biocontrol agents (BCAs), many of which have received commercial registration and are available on the market. To date, thirteen bacterial-based BCAs have been officially registered as biopesticides for managing bacterial and fungal diseases. These include several strains of *B. amyloliquefaciens* (QST 713, AH2, MBI 600, FZB24, and IT 45), *B. amyloliquefaciens* subsp. *plantarum* (strain D747), *B. firmus* (I-1582), *B. pumilus* (strain QST 2808), *B. subtilis* (strain IAB/BS03), *Pseudomonas* sp. (strain DSMZ 13134), *P. chlororaphis* (strain MA 342), *Streptomyces* K61, and *S. lydicus* (strain WYEC 108) (https://food.ec.europa.eu/plants/pesticides/eu-pesticides-database_en, accessed on 1 June 2022)

In the present study, 9 bacterial strains were screened for their potential to work as a biocontrol agent. The bacterial strains used in this study were: N1, N4 and N8 identified as *Bacillus* sp.; N2, N3 and N9 as *B. subtilis;* and the remaining (N5, N6 and N7) were *B. amyloliquefaciens*. *Bacillus* sp. is a well-documented and extensively studied bacterial species, which is present in the rhizospheric soil and has a beneficial effect on the growth of the plants. In this study, the bacterial isolate N6 *(B. amyloliquefaciens*) and N8 (*Bacillus* sp) isolates were found to show maximum beneficial effect on the germination and growth of the *Arachis hypogaea*. There are various mechanisms responsible for biopesticidal and growth-promoting activities of the bacterial isolates. The bacterial isolates secrete volatile and non-volatile secondary metabolites that are responsible for biopesticidal and growth-promoting activities. The noteworthy characteristic of *Bacillus* spp. They lie in their capacity to generate a diverse range of bioactive compounds that hold value for agricultural

purposes. This includes the production of metabolites exhibiting antimicrobial properties, surface-active agents, and involvement in triggering plant defense responses (McSpadden, 2004; Ongena and Jacques, 2008). Numerous studies have demonstrated that volatile organic compounds (VOCs) and certain lipopeptides, such as fengycin and surfactin, play a crucial role in eliciting immune responses of plants (Ongena *et al*., 2007; Cawoy *et al*., 2015). For example, *B. amyloliquefaciens* FZB42 produces secondary metabolites like surfactin, fengycin, and bacillomycin D, which contribute to the activation of plant defense genes and help in reducing the bottom rot in lettuce (Chowdhury *et al*., 2015). In another case, Bacillus subtilis OTPB1 increased the levels of growth hormones and defense-related enzymes in tomatoes, providing protection against both early and late blight (Chowdappa *et al.,* 2013). Similarly, *B. subtilis* OTPB1 has been shown to boost growth hormones and defense-related enzymes in tomatoes, offering protection against early and late blight (Chowdappa *et al*., 2013). In this context, both volatile and non-volatile secondary metabolites produced by various bacterial isolates have demonstrated biopesticidal effects against *S. rolfsii*.

Bacillus species are also known for their production of hydrolytic enzymes, including chitinases, chitosanases, glucanases, cellulases, lipases, and proteases, which degrade fungal and bacterial cell walls, thereby suppressing plant pathogens. For instance, a protease from *B. amyloliquefaciens* SP1 has been found to effectively control *Fusarium oxysporum* (Guleria *et al*., 2016). Additionally, the activity of hydrolase enzymes, such as chitinase, cellulase, protease, and glucanase, has been recognized as a key factor in *B. velezensis* for regulating gray mold of peppers, which is initiated by *Botrytis cinerea*. In this study, all the isolates have also been shown to reduce the growth of the pathogen. However, this study did not provide any mechanism to show the effect of bio-controls over pathogen growth or their effect on seed germination and plant growth. The bio-control isolates have shown an increase in seed germination, improved shoot length, reduced wilting and improved root growth. In comparison to the control, the seeds that were treated with biocontrol have improved plant height, root nodules, root dry weight and number of secondary roots. The time-dependent wilting of the plants that were treated with biocontrols was reduced in comparison to the control. Among all the 9 isolates that were tested for biocontrol potential, N6 and N9 were found to have the most potential.

Conclusion

This study evaluated the biocontrol potential of nine bacterial isolates (N1-N9) against *S. rolfsii*, the causative agent of stem rot disease in *Arachis hypogaea* (groundnut). Through a series of *in-vitro* and *in-vivo* experiments, isolates N6 (*B. amyloliquefaciens*) and N8 (*Bacillus* sp.) consistently demonstrated superior antagonistic activity, with isolate N6 emerging as the most effective across multiple parameters, including mycelial growth inhibition, sclerotial germination suppression, improved seed germination, and enhanced plant growth traits. The study confirmed that volatile organic compounds (VOCs) and non-volatile secondary metabolites produced by these isolates play a key role in inhibiting pathogen growth. Additionally, significant improvements in plant height, root architecture, dry weight, and nodule formation were recorded in biocontrol-treated seeds compared to the control, reflecting strong plant growth-promoting traits. While the exact molecular mechanisms underlying these effects remain unexplored, the findings suggest that the bioactive compounds secreted by these bacterial isolates likely contribute to disease suppression and physiological stimulation of the host plant. These results provide a promising eco-friendly alternative to chemical fungicides and offer a sustainable strategy for managing stem rot disease in groundnut. Future work should focus on molecular characterization of the bioactive compounds and field validation of these isolates for commercial-scale application.

Acknowledgment

Riddhi H. Rajyaguru is grateful to the Indian Council of Agricultural Research-Indian Institute of Groundnut Research, Junagadh, for providing laboratory facilities for the research.

Conflict of interest statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Author Contribution

Riddhi H. Rajyaguru conducted all the experiments. Thirumalaisamy PP and Jignasha T Thumar conceptualized the study. Neelam M Nathani, Chandrashekar Mootapally, and Manju Shri collaborated on writing and improving the manuscript.

References:

Abd-Allah, E.F. and El-Didamony, G. 2007. Effect of seed treatment of Arachis hypogaea with Bacillus subtilis on nodulation in biocontrol of southern blight (Sclerotium rolfsii) disease. Phytoparasitica 35(1): 8-12.

Adhilakshmi, M., Latha, P., Paranidharan, V., Balachandar, D., Ganesamurthy, K. and Velazhahan, R. 2014. Biological control of stem rot of groundnut (Arachis hypogaea L.) caused by Sclerotium rolfsii Sacc. with actinomycetes. Arch. Phytopathol. Plant Protec. 47(3): 298-311.

Alvarez, F., Castro, M., Príncipe, A., Borioli, G., Fischer, S., Mori, G., & Jofre, E. (2012). The plant-associated *Bacillus amyloliquefaciens* strains MEP218 and ARP23 capable of producing the cyclic lipopeptides iturin or surfactin and fengycin are effective in biocontrol of sclerotinia stem rot disease. Journal of applied microbiology, 112(1), 159-174.

Biswas, K.K. and Sen, C. 2000. Management of stem rot of groundnut caused by Sclerotium rolfsii through Trichoderma harzianum. Indian Phytopathol. 53(3): 290-295.

Buensanteai, N., Yuen, G. Y., & Prathuangwong, S. (2008). The biocontrol bacterium *Bacillus amyloliquefaciens* KPS46 produces auxin, surfactin and extracellular proteins for enhanced growth of soybean plant. Thai J Agric Sci, 41(3-4), 101-116.

Cawoy, H.; Debois, D.; Franzil, L.; De Pauw, E.; Thonart, P.; Ongena, M. Lipopeptides as main ingredients for inhibition of fungal phytopathogens by Bacillus subtilis/amyloliquefaciens. Microb. Biotechnol. 2015, 8, 281–295.

Chang, Y., Dong, Q., Zhang, L., Goodwin, P.H., Xu, W., Xia, M., Zhang, J., Sun, R., Wu, C., Wu, K. and Xu, S., 2025. Peanut growth promotion and biocontrol of blight by Sclerotium rolfsii with rhizosphere bacterium, Bacillus siamensis YB-1632. *Agronomy*, *15*(3), p.568.

Chowdappa, P.; Kumar, S.P.M.; Lakshmi, M.J.K.; Upreti, K. Growth stimulation and induction of systemic resistance in tomato against early and late blight by Bacillus subtilis OTPB1 or Trichoderma harzianum OTPB3. Biol. Control. 2013, 65, 109–117.

Chowdhury, S.P.; Uhl, J.; Grosch, R.; Alqueres, S.; Pittroff, S.; Dietel, K.; Schmitt-Kopplin, P.; Borriss, R.; Hartmann, A. Cyclic Lipopeptides of

Bacillus amyloliquefaciens subsp. Plantarum colonizing the lettuce rhizosphere enhance plant defense responses toward the bottom rot pathogen Rhizoctonia solani. Mol. Plant Microbe Interact. 2015, 28, 984–995

Ci, D., Qin, F., Tang, Z., Zhang, G., Zhang, J., Si, T., Yang, J., Xu, Y., Yu, T., Xu, M. and He, K., 2023. Arbuscular Mycorrhizal Fungi Restored the Saline–Alkali Soil and Promoted the Growth of Peanut Roots. *Plants*, *12*(19), p.3426.

Doley, K., Jite, P.K. 2013. Management of stem-rot of groundnut (Arachis hypogaea L.) cultivar in field. Not. Sci. Biol. 5(3): 316-324.

Dunlap, C. A., Bowman, M. J., & Schisler, D. A. (2013). Genomic analysis and secondary metabolite production in *Bacillus amyloliquefaciens* AS 43.3: a biocontrol antagonist of Fusarium head blight. Biological Control, 64(2), 166-175.

Fridlender, M., Inbar, J., & Chet, I. (1993). Biological control of soil-borne plant pathogens by a β-1, 3 glucanase-producing Pseudomonas cepacia. Soil Biology and Biochemistry, 25(9), 1211-1221.

Ganesan, S., Kuppusamy, G.R. and Sekar, R. 2007. Integrated management of stem rot disease (Sclerotium rolfsii) of groundnut (Arachis hypogaea L.) using Rhizobium and Trichoderma harzianum (ITCC-4572). Turk. J. Agric. For. 31: 103-108.

Guleria, S.; Walia, A.; Chauhan, A.; Shirkot, C.K. Molecular characterization of alkaline protease of *Bacillus amyloliquefaciens* SP1 involved in biocontrol of Fusarium oxysporum. Int. J. Food Microbiol. 2016, 232, 134–143.

Jadon, K.S., Thirumalaisamy, P.P., Kumar, V., Koradia, V.G. and Padavi, R.D., 2015. Management of soil borne diseases of groundnut through seed dressing fungicides. Crop Protection, 78, pp.198-203.

Krishna Kishore, G., Pande, S., Narayana Rao, J. and Podile, A.R. 2005. Pseudomonas aeruginosa inhibits the plant cell wall degrading enzymes of Sclerotium rolfsii and reduces the severity of groundnut stem rot. European J. Plant Pathol. 113: 315-320.

Krishnamurthy, K., & Gnanamanickam, S. S. (1998). Biological Control of Rice Blast by Pseudomonas fluorescensStrainPf7–14: Evaluation of a Marker Gene and Formulations. Biological control, 13(3), 158-165.

Lamichhane, J.R.; Dachbrodt-Saaydeh, S.; Kudsk, P.; Messéan, A. Toward a reduced reliance on conventional pesticides in European agriculture. Plant Dis. 2016, 100, 10–24.

Latha, P., Anand, T., Prakasam, V., Jonathan, E.I., Paramathma, M., Samiyappan, R. 2011. Combining Pseudomonas, Bacillus and Trichoderma strains with organic amendments and micronutrient to enhance suppression of collar and root rot disease in physic nut. Appl. Soil Ecol. 49: 215-223.

Luduena, L. M., Taurian, T., Tonelli, M. L., Angelini, J. G., Anzuay, M. S., Valetti, L., ... & Fabra, A. I. (2012). Biocontrol bacterial communities associated with diseased peanut (Arachis hypogaea L.) plants. European journal of soil biology, 53, 48-55.

Manjula, K., Krishna Kishore, G., Girish, A.G. and Singh, S.D. 2004. Combined application of Pseudomonas fluorescens and Trichoderma viride has an improved biocontrol activity against stem rot in groundnut. Plant Pathol. J. 20(1): 75-80.

McSpadden Gardener, B.B. Ecology of Bacillus and Paenibacillus spp. in agricultural systems. Phytopathology 2004, 94, 1252–1258.

Neelipally, R.T.K.R., Anoruo, A.O. and Nelson, S., 2020. Effect of Co-Inoculation of Bradyrhizobium and Trichoderma on growth, development, and yield of Arachis hypogaea L. (Peanut). *Agronomy*, *10*(9), p.1415.

Oldenburg E, Hoppner F, Weinert J (2005) Distribution of deoxynivalenol in Fusarium-infected forage maize. Mycotoxin Res 21:196–199

Ongena, M.; Jacques, P. Bacillus lipopeptides: Versatile weapons for plant disease biocontrol. Trends Microbiol. 2008, 16, 115–125.

Ongena, M.; Jourdan, E.; Adam, A.; Paquot, M.; Brans, A.; Joris, B.; Arpigny, J.L.; Thonart, P. Surfactin and fengycin lipopeptides of Bacillus subtilis as elicitors of induced systemic resistance in plants. Environ. Microbiol. 2007, 9, 1084–1090.

Onobri, A. 2007. Routine statistical analysis of field experiments by using an excel extension. Proceedings 6th national conference Italian Biometric Society: "La statistica nelle science della vita e dell and I ante" Pisa, 20–22 June, pp. 93–96

Pandey, M. K., Kumar, R., Pandey, A. K., Soni, P., Gangurde, S. S., Sudini, H. K., & Varshney, R. K. (2019). Mitigating aflatoxin contamination in groundnut through a combination of genetic resistance and post-harvest management practices. Toxins, 11(6), 315.

Poosapati, S., Ravulapalli, P.D., Tippirishetty, N., Vishwanathaswamy, D.K. and Chunduri, S. 2014. Selection of high temperature and salinity tolerant Trichoderma isolates with antagonistic activity against Sclerotium rolfsii. SpringerPlus 3: 641.

Rajyaguru, R. H., Thirumalaisamy, P. P., Patel, K. G., & Thumar, J. T. (2017). Biochemical basis of genotypic and bio-agent induced stem rot resistance in groundnut. Legume Research: An International Journal, 40(5).

Sanjel, S., Colee, J., Barocco, R.L., Dufault, N.S., Tillman, B.L., Punja, Z.K., Seepaul, R. and Small, I.M., 2024. Environmental factors influencing stem rot development in peanut: Predictors and action thresholds for disease management. *Phytopathology®*, *114*(2), pp.393-404.

Saravanakumar, K., Li, Y., Yu, C., Wang, Q. Q., Wang, M., Sun, J., ... & Chen, J. (2017). Effect of Trichoderma harzianum on maize rhizosphere microbiome and biocontrol of Fusarium Stalk rot. Scientific reports, 7(1), 1771.

Turner, J. T., & Backman, P. A. (1991). Factors relating to peanut yield increases after seed treatment with Bacillus subtilis.

Wang, X., & Liang, G. (2014). Control efficacy of an endophytic *Bacillus amyloliquefaciens* strain BZ6-1 against peanut bacterial wilt, Ralstonia solanacearum. BioMed research international, 2014(1), 465435.

Ye, W. Q., Sun, Y. F., Tang, Y. J., & Zhou, W. W. (2021). Biocontrol potential of a broad-spectrum antifungal strain Bacillus amyloliquefaciens B4 for postharvest loquat fruit storage. *Postharvest Biology and Technology*, 174, 111439.

Yuan, J., Yu, L., Ling, N., Raza, W., Shen, Q., & Huang, Q. (2015). Plant-growth-promoting traits and antifungal potential of the *Bacillus amyloliquefaciens* YL-25. Biocontrol Science and Technology, 25(3), 276-290.