

## Antagonistic nodule endophytic bacteria effective against *Sclerotium rolfsii* causing stem rot disease in groundnut (Given)

### ABSTRACT

Groundnut is a major oilseed crop grown throughout the world. The crop is constrained by several biotic and abiotic stresses, among which stem rot disease (of groundnut crop) caused by *Sclerotium rolfsii* poses significant threat to groundnut production. The management of stem rot disease using chemical methods has adverse health and ecological impacts. Environmentally friendly alternatives such as the use of microbial antagonists including bacterial endophytes is a sustainable approach. In this context, (the) this study explored the potential of nodule endophytic bacteria of groundnut for antagonism against *S. rolfsii*. A survey was conducted in major groundnut growing regions of Telangana during *rabi*, 2023. Eight different isolates of *S. rolfsii* and twenty 20 nodule endophytic bacterial isolates were isolated from the infected and healthy groundnut plants, respectively. *S. rolfsii* isolate GNS1 was the most virulent of all with the lowest incubation period and days to permanent wilting. Among the 20 bacterial endophytes, isolates GNEB13 and GNEB6 were the most effective with highest mycelial inhibition of *S. rolfsii* under *in vitro* conditions. These isolates have the potential to be evaluated under field conditions for sustainable management of stem rot disease in groundnut.

**KEYWORDS:** Groundnut, dual culture technique, endophytes, nodule endophytic bacteria, stem rot and *Sclerotium rolfsii*

### INTRODUCTION

Groundnut (*Arachis hypogaea* L.), also commonly known as peanut, is the third most important oilseed crop grown across the world. India is the second leading country of groundnut after China both in terms of area and production (Meena *et al.*, 2024). The production is estimated at 7.82 million tonnes with majority of the crop cultivated in the states of Gujarat, Rajasthan, Andhra Pradesh, Tamil Nadu, Karnataka and Telangana ([www.agricoop.gov.in](http://www.agricoop.gov.in)). Groundnut production is constrained by many abiotic and biotic stresses including bacterial, fungal and viral diseases. Among the fungi, *Sclerotium rolfsii* (sacc.) causing stem rot disease is a destructive soil-borne pathogen affecting groundnut yields throughout the world including India with yield losses of up to 50% (per cent) (Ashok Kumar and Ramadevi, 2024). It is a ubiquitous, polyphagous soil-borne pathogen with an extensive host range, affecting over 500 different plant species and is (Desai and Scholsser, 1999) and is characterized by rapid mycelial growth and resilient sclerotia, which result in considerable economic losses (Cilliers *et al.*, 2003). These features also render the management of stem rot difficult. Even though use of chemicals is widely adopted and is the growers' first

choice to manage the disease, frequent and indiscriminate use poses significant health and ecological risks.

Sustainable management of stem rot using biological control is a promising eco-friendly alternative to fungicides. The potential of rhizosphere microorganisms and endophytes has been reported in managing *S. rolf sii* (Akash *et al.*, 2022; Motlagh *et al.*, 2022). Endophytic bacteria promote plant growth by producing auxins, phytohormones, nitrogen fixation, solubilizing phosphate, releasing ammonia etc. and also provide protection against phytopathogens by volatile metabolites including hydrogen cyanide, ammonia, siderophores production and anti-oxidant enzymes etc. (Saini *et al.*, 2015; Nagpal *et al.*, 2021). Nodules in legume plants such as groundnut contain diverse endophytic bacteria including rhizobial and non-rhizobial endophytes (NRE) (Hossain *et al.*, 2023). While, the former traditionally belong to the genera *Azorhizobium*, *Bradyrhizobium*, *Ensifer*, *Mesorhizobium* and *Rhizobium* NRE include *Aminobacter*, *Aerobacter*, *Bacillus*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Staphylococcus* etc. (De Meyer *et al.*, 2015). Despite the potential of endophytes in stress management and adequate knowledge on the nodule endophytes (Preyanga *et al.*, 2021) studies on bio-control potential of nodule endophytic bacteria against stem rot disease in groundnut crop is limited. Therefore the present study aimed to exploit the nodule endophytic bacteria with antagonistic ability as potential biocontrol agents for the management of stem rot disease in groundnut.

## **Materials and Methods**

### **Survey for collection of stem rot infected plants and healthy roots**

A roving survey was conducted in major groundnut growing areas of Telangana viz., Mahabubnagar and Nagarkurnool districts during *rabi*, 2023. At 30-45 days after sowing, stem rot infected plant samples and healthy root samples with nodules were collected for isolation of the pathogen (*Sclerotium rolf sii*) and endophytic bacteria respectively during 30-45 days after sowing. Stem rot infected plants were identified based on the external signs and symptoms such as the presence of white mycelial growth, sclerotia, lesion on the stem, wilting drying or dead plants. Healthy plants were gently uprooted without damaging the root system for isolation of nodule endophytic bacteria. The plants were washed in tap water to remove adhering soil particles, packed in air-tight pouches and brought to the laboratory (Hassen *et al.*, 2018). The samples were carried in ice box during transportation to the laboratory.

### **Isolation and characterization of the pathogen**

The pathogen *S. rolf sii* was isolated from the stems of stem rot disease infected groundnut plants with white mycelial growth on the collar region on potato dextrose agar (PDA) medium by tissue

segment method (Rangaswami and Mahadevan, 1999). Small tissue bits of 0.5 – 1 cm were cut from the infected collar region along with some healthy tissues using a sterile scalpel. The tissue bits were surface sterilized using 1 per cent sodium hypochlorite solution for 30 seconds followed by washing the min sterile distilled water for three times to remove the residual chemical. They were placed in Petri dishes with solidified PDA and incubated at 25±2°C. The plates were observed periodically for the growth of the pathogen. Axenic cultures of the pathogen was obtained by single hyphal tip method and maintained on PDA throughout the investigation. Observations on the time taken by mycelial growth to cover the entire plate were recorded for each isolate. Further, colony characters such as colony colour, colony type, growth type along with sclerotial characters such as the time required for production and maturation of the sclerotia, number of sclerotia produced in plate, sclerotial colour, sclerotial weight per 100 sclerotia and sclerotial size were recorded for each isolate.

### **Pathogenicity of the isolates**

The eight isolates obtained from surveyed locations were evaluated for their pathogenicity in pot culture experiment using the susceptible groundnut variety Kadiri-6 to identify the most virulent isolate for further studies (Akash *et al.*, 2022). The uniform size pots were covered measuring 20 ×25 cm and filled with sterilized pot mixture comprising of soil, sand and vermicompost in 2:1:1 ratio. Groundnut seeds were surface sterilized using 0.1 per cent sodium hypochlorite and five seeds were sown per pot and finally three seedlings were maintained in each pot. The pathogen isolates were mass multiplied on sterilized sorghum grains pre-soaked overnight in 2 per cent sucrose (Upadhyay and Mukhopadhyay, 1986). Thirty day old seedlings were inoculated with 25g inoculum per pot by spreading the inoculum on the surface of the soil. An untreated control without the inoculum was also maintained. Observations on incubation period (IP) and days to permanent wilting (DPW) were made from next day after inoculation. The pathogen was re-isolated from symptomatic plant tissues and compared to the original isolate for conformity.

### **Isolation and characterization of nodule endophytic bacteria**

The endophytic bacteria from root nodules of groundnut were isolated based on the method given by (Preyanga *et al.*, 2021). The nodules were separated from the roots carefully avoiding any damage and were surface-sterilized using 3 per cent sodium hypochlorite for 30 seconds. The nodules were rinsed for five times in sterile distilled water to remove excess chemicals. They were crushed using a sterile glass rod and were diluted in sterile distilled water up to 10<sup>-12</sup> and 0.1 ml of each dilution was spread over Petri dishes containing nutrient agar with three replications. The plates were incubated at 28±2°C for 24–48h. Bacteria were evaluated for their colony/cultural characteristics viz., shape, margin, elevation, size, texture, appearance, pigmentation and optical property. Gram staining was performed on bacteria that had been cultured for 24h. Selected

bacterial colonies were further purified through repeated streaking on the same medium. These isolates were stored in 60 per cent glycerol stocks at -80 °C for future experiments.

### ***In vitro* efficacy of nodule bacterial endophytes against *S. rolf sii***

The nodule endophytic bacteria were screened *in vitro* for their antagonism against the stem rot pathogen *S. rolf sii* by dual culture technique (Dennis and Webster, 1971; Akash *et al.*, 2022). Loopful of 24h old pure cultures of test isolate was streaked 1cm away from the periphery of the PDA plates. Thereafter 5 mm mycelial disc of 5-day old culture of *S. rolf sii* was placed at the opposite end and the plates were incubated at 25±2°C. A control plate with only *S. rolf sii* was also maintained. Observations were made when full growth was achieved in the control plate. The mycelial growth of the pathogen was measured in each Petri dish separately and expressed in mm. The per cent inhibition of the mycelial growth of the pathogen by different test isolates was calculated (Vincent, 1947).

$$I = \frac{C-T}{C} \times 100$$

where *I* is the per cent inhibition of mycelial growth over control; *C* is the radial growth of the pathogen in control (mm); *T* is the radial growth of the pathogen in treatment (mm)

### **Results and Discussion**

The survey covered 12 groundnut fields in 11 villages covering five Mandals of Nagarkurnool and Mahbubnagar districts of Telangana (Table 1). Solo cropping of groundnut variety Kadiri-6 was followed by the farmers in surveyed locations. The disease incidence of stem rot in the surveyed fields ranged between 10 and 28 per cent with a mean of 19.04 percent (Table 1) (Write means in Table by adding column). Highest incidence was observed in Venkatapur village in Nagarkurnool district of Telangana state followed by Khanapur village. The lowest disease incidence was observed in Achampet village. Akash *et al.*, (2022a) reported stem rot incidence of 7.84 to 20.63 per cent in groundnut. This is almost nearer to the disease incidence reported in our study. Compared to this study, the pathogen *S. rolf sii* resulted in higher disease incidence of 35 per cent in brinjal fields of Karnataka district. These differences might be due to the the differences in the virulence of the pathogen across locations, edaphic factors, climate and agronomic practices (Kirankumar *et al.*, 2024).

**Table 1: Data collected during sample collection from groundnut growing fields of Nagarkurnool and Mahbubnagar districts during Rabi 2023-2024**

Name of Mandal and village	Field location	Variety grown	Stage of the	PDI of <i>Sclerotium</i>
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					<b>crop (DAS)</b>	<b>stem rot (%)</b>
<b>Mandal</b>	<b>Village</b>	<b>Latitude</b>	<b>Longitude</b>			
Bijinepally	Khanapur	16.507412°	78.227492°	Kadiri-6	50	25
Bijinepally	Khanapur	16.505401°	78.222516°	Kadiri-6	45	18
Bijinepally	Vattem	16.505393°	78.222585°	Kadiri-6	45	22
Achampet	Achampet	16.664522°	78.55597°	Kadiri-6	55	10
Nagarkurnool	Peddapuram	16.68066°	78.563712°	Kadiri-6	40	12
Nagarkurnool	Malkapur	16.680649°	78.563698°	Kadiri-6	45	22
Nagarkurnool	Venkatapur	16.722328°	78.566715°	Kadiri-6	45	28
Veldanda	Veldanda	16.51448°	78.250468°	Kadiri-6	50	16.5
Veldanda	Peddapur	16.51645513°	78.23946526°	Kadiri-6	45	12
Veldanda	Kotra	16.5315°	80.4222°	Kadiri-6	45	20
Jadcherla	Vallur	16.712981°	78.433049°	Kadiri-6	60	25
Jadcherla	Kodgal	16.712997°	78.433001°	Kadiri-6	55	18

### **Identification and characterization of the pathogen**

The pathogen was identified as *S. rolfisii* based on the mycelial and sclerotial characters (Barnett and Hunter, 1972). A total of 8 pure cultures *S. rolfisii* were obtained from the collected stem rot infected groundnut samples. All the isolates of *S. rolfisii* produced white cottony mycelial growth on PDA media characterized by profuse to highly profuse colony growth (Table 2)

**Table 2: Culture characteristics of different isolates of *S. rolfii* on PDA media**

Isolate ID	Color	Colony type	Growth type	Time to cover entire plate (days) (Write sign)#
GNSR 1	White	Raised at ends	Highly profuse	3.67 <sup>a</sup>
GNSR 2	White	Raised at ends	Highly profuse	5.67 <sup>d</sup>
GNSR 3	White	Flat	Profuse	5.33 <sup>c</sup>
GNSR 4	White	Raised at ends	Highly profuse	5.67 <sup>d</sup>
GNSR 5	White	Raised at ends	Highly profuse	4.67 <sup>b</sup>
GNSR 6	Dull white	Flat	Highly profuse	5.33 <sup>c</sup>
GNSR 7	Dull white	Raised at ends	Highly profuse	6.67 <sup>e</sup>
GNSR 8	Dull white	Raised at ends	Profuse	5.33 <sup>c</sup>

(Write sign)# Values are expressed in means of three replicates; Values in columns with the same letters after them indicate insignificant differences at the 5% significance level

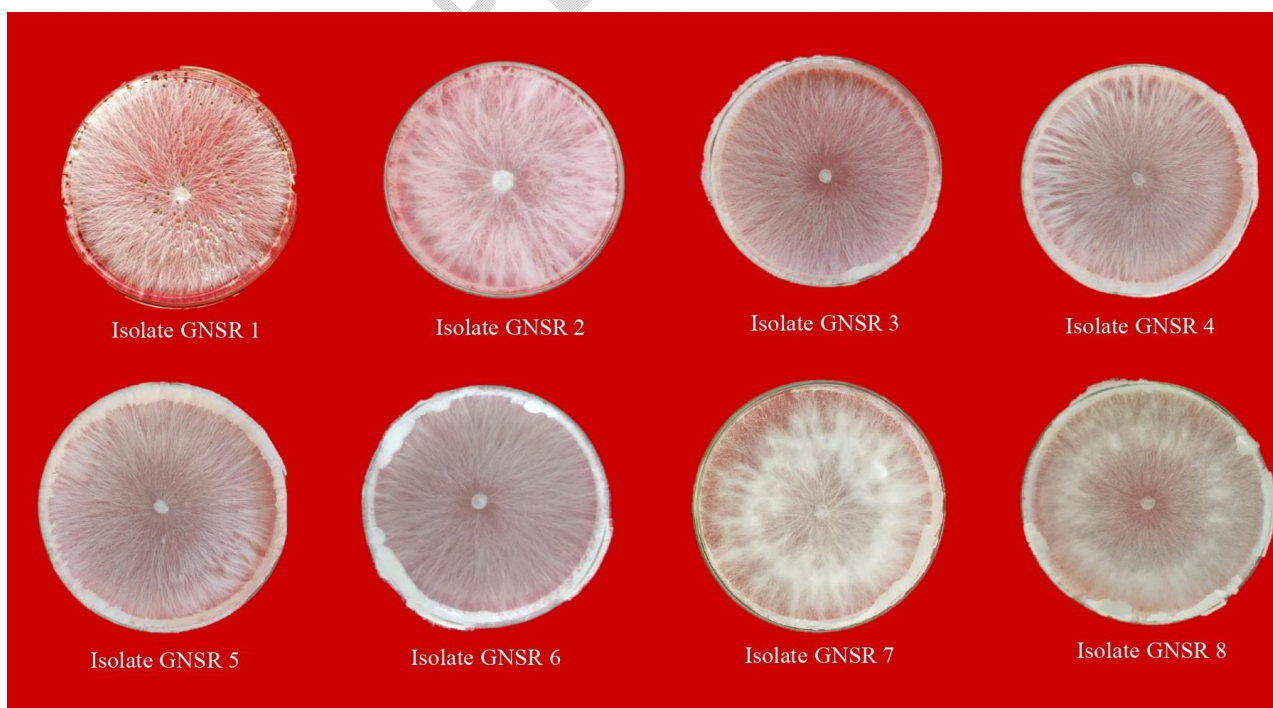
Further it was observed that the isolates GNSR 3 and GNSR 8 with profuse growth appeared flat and raised respectively. The colonies of the isolates GNSR 1, GNSR 2, GNSR 4, GNSR 5, GNSR 6 and GNSR 7 which produced highly profuse growth were raised at the ends. The isolates took an average 3.67 (GNSR 1) to 6.67 days (GNSR 7) to cover the entire plate with white mycelial growth. Similar results were reported by Akash *et al.*, (2022a) and Kumar *et al.*, (2014) who also reported on *S. rolfii* isolates taking 3-5 days to cover the entire plate. In the current study, isolate GNSR 1 covered the entire Petri dish in 3.67 days and hence was considered as fast growing isolate. The isolates also differed in their sclerotial characteristics (Table 3). The average number of sclerotia produced ranged from 6.33 (GNSR 6) to 16.67 (GNSR 1). The average size of sclerotia produced by the isolates ranged from 1.14 (GNSR 3) to 1.61mm (GNSR 1). The average weight of 100 sclerotia ranged from 0.032 (GNSR 6) to 0.126g (GNSR 5). Colour of the mycelium varied from light brown to dark brown (Table 3)

**Table 3: Sclerotial characteristics of *S. rolfii* on Potato dextrose agar medium**

Isolate ID	Time required to (Days)	Arrangement	Colour	Weight (g/100 sclerotia)	Number/ Plate	Size (mm)
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	<b>Produce</b>	<b>Mature</b>					
<b>GNSR 1</b>	16.67 <sup>g</sup>	20.33 <sup>f</sup>	Scattered	Dark brown	0.039 <sup>b</sup>	107.3 <sup>cd</sup>	1.61 <sup>e</sup>
<b>GNSR 2</b>	11.33 <sup>d</sup>	14.67 <sup>c</sup>	Scattered	Dark brown	0.051 <sup>d</sup>	49 <sup>b</sup>	1.52 <sup>d</sup>
<b>GNSR 3</b>	8.33 <sup>b</sup>	13 <sup>b</sup>	Peripheral	Dark brown	0.046 <sup>c</sup>	117.67 <sup>e</sup>	1.14 <sup>a</sup>
<b>GNSR 4</b>	15.33 <sup>f</sup>	17 <sup>d</sup>	Scattered	Dark brown	0.067 <sup>f</sup>	138.67 <sup>f</sup>	1.57 <sup>de</sup>
<b>GNSR 5</b>	9 <sup>c</sup>	12 <sup>a</sup>	Peripheral	Light brown	0.126 <sup>h</sup>	104.33 <sup>c</sup>	1.45 <sup>c</sup>
<b>GNSR 6</b>	6.33 <sup>a</sup>	11.67 <sup>a</sup>	Scattered	Dark brown	0.032 <sup>a</sup>	110.33 <sup>d</sup>	1.21 <sup>b</sup>
<b>GNSR 7</b>	12.67 <sup>e</sup>	16.33 <sup>d</sup>	Scattered	Dark brown	0.062 <sup>e</sup>	104.67 <sup>c</sup>	1.23 <sup>b</sup>
<b>GNSR 8</b>	15.68 <sup>f</sup>	18 <sup>e</sup>	Scattered	Light brown	0.094 <sup>g</sup>	39 <sup>a</sup>	1.42 <sup>c</sup>

(Same as above Table) Values are expressed in means of three replicates; Values in columns with the same letters after them indicate insignificant differences at the 5% significance level



**Fig. 1** Isolates of pathogen *Sclerotium rolfsii*

Variability among the pathogen isolates was reported by Le *et al.*, (2012), Thilagavathi *et al.*, (2013), Akram *et al.*, (2015) and more recently Akash *et al.*, (2022a) in terms of colony morphology, mycelial growth rate, sclerotia formation, number, weight and color, time for maturation etc. This might be because the isolates were collected from different regions across India with different edaphic, climatic and agronomic factors (Kirankumar *et al.*, 2024). Sclerotial characteristics like density, viability, time taken for maturation are influenced by several soil type, soil pH etc. (Sen *et al.*, 2023). *S. rolfsii* has a wide-host range and hence factors like cropping systems followed by the farmers and crop residues might result in the variability of the isolates. For instance paddy-maize and paddy-cucurbits cropping system is suppressive towards *S. rolfsii* and paddy-legume or groundnut based cropping system as seen in the current study is conducive towards *S. rolfsii* resulting in higher sclerotial number and density.

#### Pathogenicity of the isolates

All the isolates showed cent per cent disease incidence in groundnut however they differed with respect to the IP and DPW (Table 4). The IP ranged from 4.5 (isolate GNSR 1) to 7 (isolate GNSR 5) days and DPW ranged from 12.5 (isolate GNSR 1) to 21.5 (isolate GNSR 7) days. The isolate GNS 1 with lowest IP and DPW was identified as the most virulent isolate among all the 11 evaluated isolates and hence was further used in the study.

**Table 4: Incubation period and days to permanent wilting of the isolates of *S. rolfsii* on groundnut variety Kadiri-6 (K6)**

Isolate ID	Incubation period (dpi)	Days to permanent wilting (dpi)
GNSR 1	4.50 <sup>a</sup>	12.50 <sup>a</sup>
GNSR 2	5.50 <sup>d</sup>	15.25 <sup>b</sup>
GNSR 3	5.00 <sup>c</sup>	15.00 <sup>b</sup>
GNSR 4	5.50 <sup>d</sup>	14.75 <sup>b</sup>
GNSR 5	7.00 <sup>e</sup>	18.50 <sup>d</sup>
GNSR 6	5.50 <sup>d</sup>	17.00 <sup>c</sup>
GNSR 7	5.00 <sup>c</sup>	21.50 <sup>e</sup>
GNSR 8	4.75 <sup>b</sup>	15.25 <sup>b</sup>

(Same as above) Values are expressed in means of three replicates; Values in columns with the same letters after them indicate insignificant differences at the 5% significance level

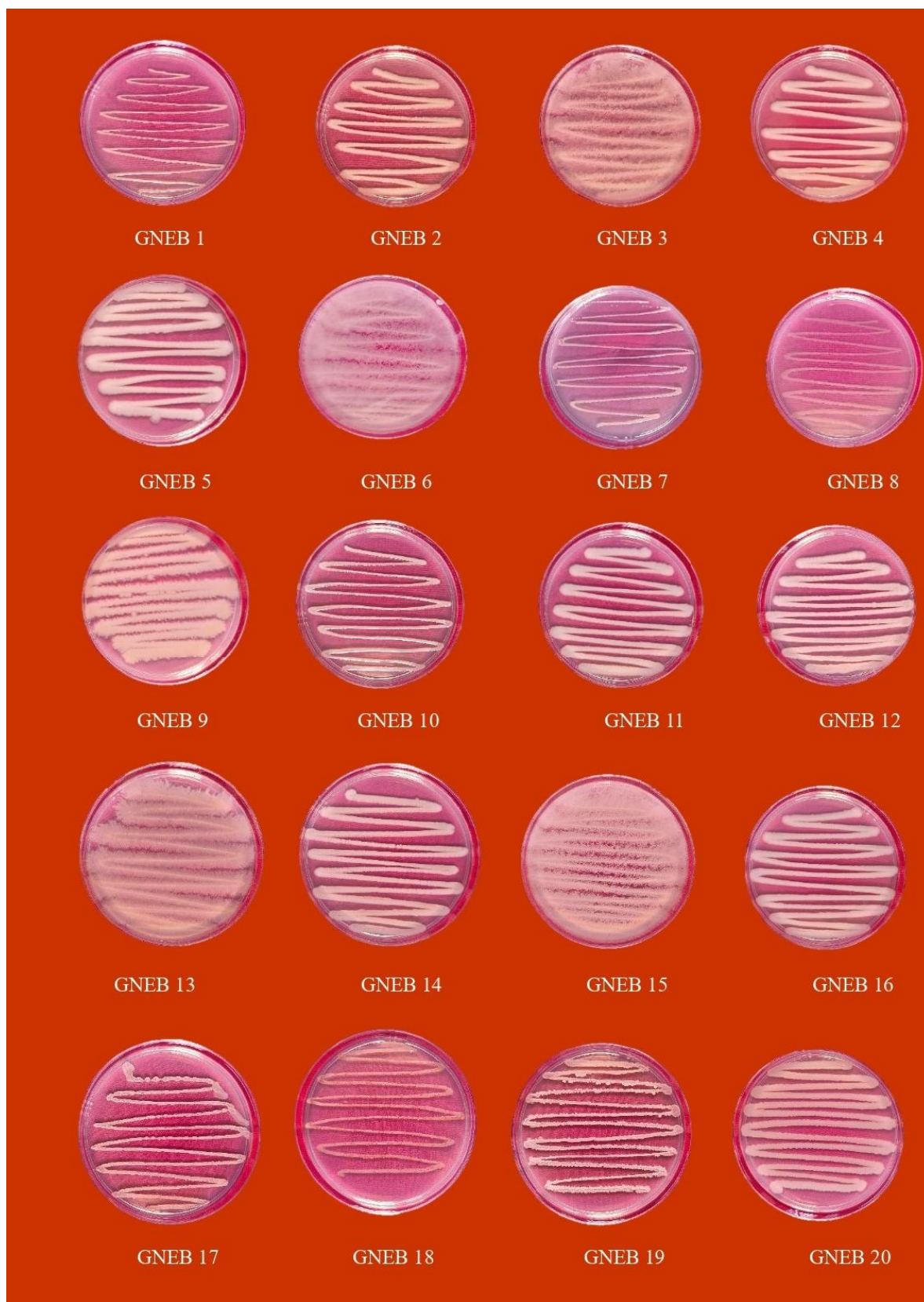
Similar values for IP and DPW ranged from 4.25 to 6.75 days and 12.25 to 21.25 days respectively were reported by Akash *et al.*, (2022a) conforming the average time taken for the pathogen to incubate and result in permanent wilting in groundnut plants. This might be because the isolates might be similar attributed to the proximity of the surveyed locations in Akash *et al.*, (2022a).

### **Isolation and characterization of nodule endophytic bacteria**

A total of 20 endophytic bacteria were isolated on nutrient media. The shape of the bacteria varied from circular to irregular with entire and wavy margins respectively. The elevation of the colonies varied from raised. Slightly raised and flat. Most of the bacteria were with the smooth and shiny colonies however some were rough in appearance. Size of the bacterial colonies varied from large, moderate, small and pinpoint. The appearance of the colonies varied from moist, butyrous, dry, mucoid and brittle. Most of the bacteria were creamy white in colour and cream in colour. However some were light yellow, light brown and transparent. In gram's staining reaction, most of the bacteria showed gram positive nature (Table 5). Similarly, in the morphological characterization of endophytic bacterial isolates, a variety of colony shapes, colors, margins, and textures were observed. The colonies exhibited circular and irregular shapes, ranging in color from off-white to white and yellowish. Their margins were either regular or wavy. Gram staining revealed that three isolates were Gram-negative, while the rest were Gram-positive (Duhan *et al.*, 2020). Colony morphology provides insight into phenotypic variation and is a critical adaptive strategy that bacteria use to endure environmental stressors. Furthermore, alterations in colony traits can indicate heightened virulence and greater resistance to antimicrobials (Sousa *et al.*, 2013). It is also commonly employed to differentiate bacterial genotypes on culture plates and serves as a valuable marker of ecological diversity (Saxer *et al.*, 2010).

**Table 5: Cultural and morphological characteristics of potential endophytic bacterial isolates**

<b>Isolate</b>	<b>Shape</b>	<b>Margin</b>	<b>Elevation</b>	<b>Size</b>	<b>Texture</b>	<b>Appearance</b>	<b>Pigment</b>	<b>Gram staining reaction</b>
GNEB 1	Circular	Entire	Raised	Large	Mucoid	Smooth	Creamy white	Positive
GNEB 2	Circular	Entire	Flat	Moderate	Mucoid	Smooth	Cream	Negative
GNEB 3	Circular	Entire	Slightly raised	Pinpoint	Dry	Rough	creamy white	Negative
GNEB 4	Irregular	Wavy	Raised	Large	Moist	Smooth	cream	Positive
GNEB 5	Circular	Entire	Flat	Large	Butyrous	Shiny	cream	Positive
GNEB 6	Irregular	Wavy	Slightly raised	Moderate	Mucoid	Rough	Creamy white	Negative
GNEB 7	Circular	Entire	Raised	Large	Mucoid	Shiny	Transparent	Positive
GNEB 8	Circular	Entire	Slightly raised	Small	Dry	Rough	Creamy white	Positive
GNEB 9	Irregular	Wavy	Flat	Small	Mucoid	Shiny	cream	Positive
GNEB 10	Circular	Entire	Slightly raised	Moderate	Moist	Smooth	Light yellow	Positive
GNEB 11	Circular	Entire	Slightly raised	Moderate	Butyrous	Smooth	Light yellow	Positive
GNEB 12	Circular	Entire	Flat	Large	Mucoid	Shiny	Creamy white	Positive
GNEB 13	Irregular	Wavy	Raised	Moderate	Brittle	Rough	Light brown	Positive
GNEB 14	Circular	Entire	Flat	Pinpoint	Mucoid	Shiny	Cream	Positive
GNEB 15	Irregular	Wavy	Raised	Large	Brittle	Rough	Cream	Positive
GNEB 16	Circular	Entire	Raised	Moderate	Butyrous	Smooth	Creamy white	Positive
GNEB 17	Circular	Entire	Slightly raised	Small	Butyrous	Smooth	Cream	Negative
GNEB 18	Circular	Entire	Raised	Moderate	Mucoid	Shiny	Creamy white	Negative
GNEB 19	Irregular	Wavy	Flat	Small	Brittle	Rough	Cream	Positive
GNEB 20	Circular	Entire	Raised	Large	Butyrous	Shiny	Light yellow	Negative



**Fig. 2 Pure cultures of endophytic bacterial isolates on nutrient agar media**

### ***In vitro* efficacy of nodule endophytic bacteria against *S. rolf sii***

All the 20 endophytic bacterial isolates were tested for their antagonism against *S. rolf sii* *in vitro* by dual culture technique. Observations were taken when the radial growth of pathogen in the culture plate was full. Among the isolates tested, isolate GNEB 13 recorded maximum inhibition of 73.3 per cent over control followed by isolate GNEB 6 (71.40%) and GNEB 16 and GNEB 15 (68.51%), followed by GNEB 3(67.70%), GNEB 4(67.40%) Minimum inhibition of 24.81 per cent over control was recorded by GNEB 19 isolate followed by GNEB 18 (25.93%). Maximum zone of inhibition was shown by the isolate GNEB 4 (23.40 mm), followed by GNEB 6 (22.50 mm) and GNEB 16 and GNEB 8 (22.0 mm). The lowest zone of inhibition was shown by the isolates GNEB 20 (2.5 mm), GNEB 1 (7.2 mm) and GNEB 15 (9.8 mm) whereas isolates GNEB 9, GNEB 14. GNEB 19 showed no inhibition zone.

Endophytes protect plant from phytopathogens and promote plant growth thereby increasing its tolerance against several biotic and abiotic stresses (Khanna et al., 2022). In the current study, 20 culturally and biochemically distinct endophytic bacteria were isolated from the root nodules of groundnut plants. Several studies reported a wide variation in the endophytic bacterial genera isolated from the roots of groundnut. Archana et al. (2020) isolated 16 bacterial endophytes from groundnut and reported isolate EGN1 as most potential against *S. rolf sii*. Akash et al. (2022b) identified most effective bacterial endophytes isolated from rhizosphere of groundnut as *Bacillus subtilis*. Maheswari et al. (2019) revealed the identity of bacterial isolates as *Pantoea agglomerans*, *B. cereus*, *B. sonorensis*, *B. subtilis*, *P. Chlororaphis* etc isolated from the roots and nodules of chickpea. Similarly, Li et al. (2023) isolated 45 bacterial strains from healthy peanut plants, with 6 showing antagonistic activity against *S. rolf sii*. Among these, *Bacillus* sp. Isolate F-1 and *Burkholderiasp.* Isolate R-11 exhibited the strongest activity, with inhibition zone widths of 20.25 mm and 15.49 mm, respectively. Koopa and Krishnaraj (2017) found that the *Pseudomonas* isolate AUDP 48 exhibited the most significant inhibition of *S. rolf sii* mycelial growth using the dual culture method. Similarly, Paramasivan *et al.*, (2019) reported six *Pseudomonas* sp. isolates tested for antagonistic activity against *S. rolf sii*, of which *P. fluorescens* (SBHRP2) achieved the highest inhibition rate of 66.36% followed by *P. fluorescens* (SBHRPF4) and *P. chlororaphis* (PA 23), which recorded inhibition rates of 65.27% and 64.77% respectively, compared to the control.

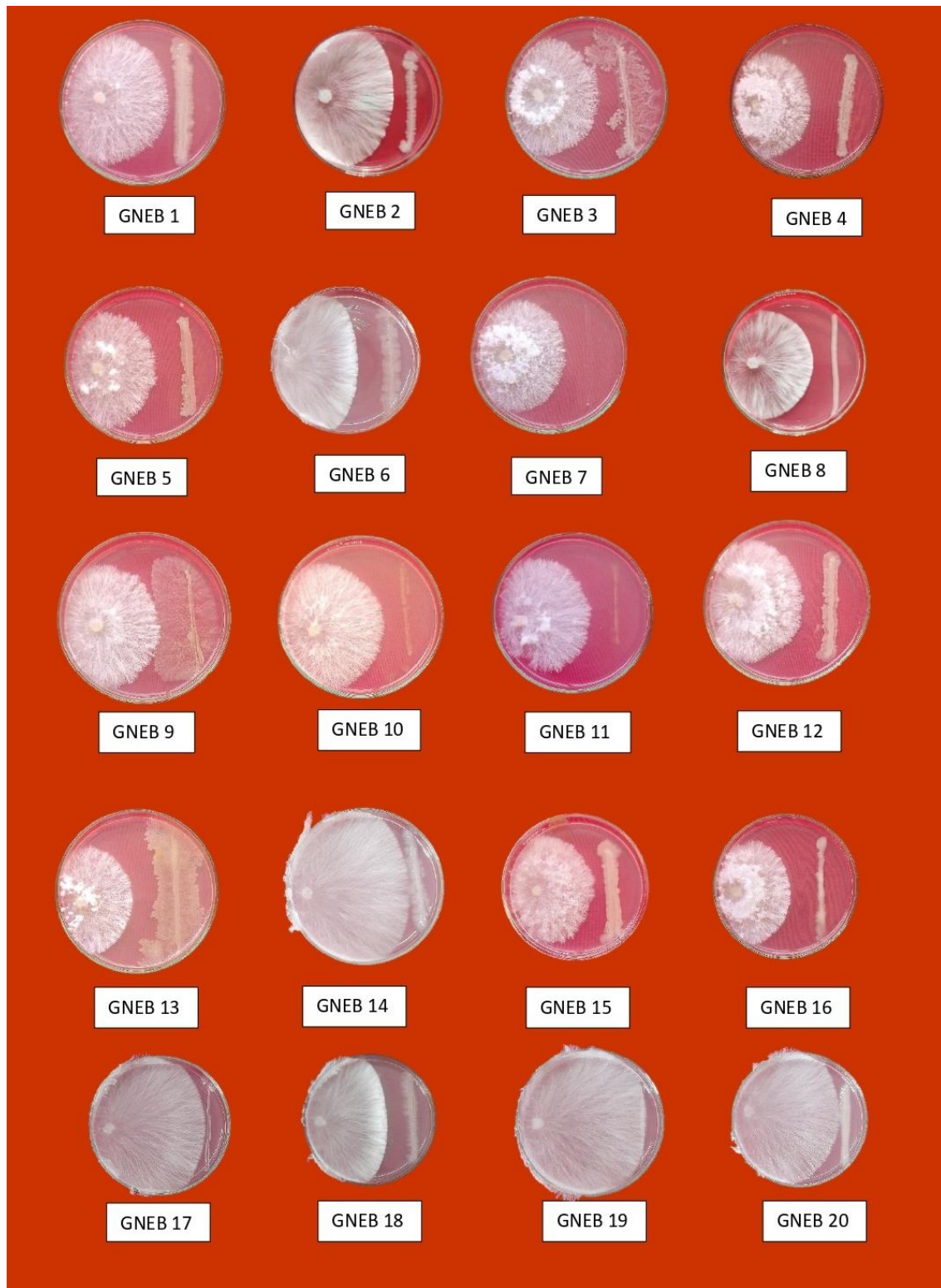
In the present study, isolates of the stem rot pathogen *S. rolf sii* and nodule bacterial endophytes were isolated from groundnut plants. The pathogen isolates were characterized for their IP and DPW and the most virulent isolate GNS1 was used for *in vitro* studies. Twenty nodule

endophytic bacterial isolates were evaluated against *S. rolf sii* isolate GNS1 by dual culture technique and two isolates GNEB13 and GNEB 6 were the most effective in inhibiting the mycelial growth of *S. rolf sii* isolate GNS1. These two isolates have the potential to be evaluated under field conditions for sustainable management of stem rot disease in groundnut.

**Table 6: Antagonistic activity of endophytic bacterial isolates in radial growth of *S. rolf sii* by dual culture technique**

Isolate	Mean (mm) add letters	% inhibition over control	Zone of inhibition (mm)
GNEB 1	35 <sup>d</sup>	61.10 <sup>i</sup>	7.20 <sup>j</sup>
GNEB 2	31.67 <sup>fg</sup>	64.81 <sup>efgh</sup>	12.50 <sup>h</sup>
GNEB 3	29 <sup>ijk</sup>	67.70 <sup>cde</sup>	16.00 <sup>g</sup>
GNEB 4	29.33 <sup>ij</sup>	67.40 <sup>cde</sup>	23.40 <sup>a</sup>
GNEB 5	31 <sup>fgh</sup>	65.50 <sup>efg</sup>	21.80 <sup>bc</sup>
GNEB 6	25.67 <sup>m</sup>	71.40 <sup>ab</sup>	22.50 <sup>b</sup>
GNEB 7	30.33 <sup>ghi</sup>	66.20 <sup>def</sup>	21.40 <sup>cd</sup>
GNEB 8	27.67 <sup>kl</sup>	69.20 <sup>bc</sup>	22.00 <sup>bc</sup>
GNEB 9	30.33 <sup>ghi</sup>	66.20 <sup>def</sup>	0 <sup>l</sup>
GNEB 10	33.67 <sup>de</sup>	62.50 <sup>hi</sup>	18.50 <sup>f</sup>
GNEB 11	32.33 <sup>ef</sup>	64.07 <sup>fgh</sup>	20.50 <sup>e</sup>
GNEB 12	33.33 <sup>e</sup>	62.90 <sup>ghi</sup>	18.00 <sup>f</sup>
GNEB 13	24 <sup>n</sup>	73.30 <sup>a</sup>	21.00 <sup>de</sup>
GNEB 14	29.67 <sup>hij</sup>	67.03 <sup>cde</sup>	0 <sup>l</sup>
GNEB 15	28.33 <sup>l</sup>	68.51 <sup>bcd</sup>	9.80 <sup>i</sup>
GNEB 16	28.33 <sup>kl</sup>	68.51 <sup>bcd</sup>	22.00 <sup>bc</sup>
GNEB 17	39 <sup>c</sup>	35.93 <sup>a</sup>	0 <sup>l</sup>
GNEB 18	38.67 <sup>c</sup>	25.93 <sup>k</sup>	21.50 <sup>cd</sup>
GNEB 19	41.33 <sup>b</sup>	24.81 <sup>k</sup>	0 <sup>l</sup>
GNEB 20	46.67 <sup>a</sup>	26.67 <sup>k</sup>	2.50 <sup>k</sup>
Control	90	0	0

(Same) Values are expressed in means of three replicates; Values in columns with the same letters after them indicate insignificant differences at the 5% significance level



**Fig. 3** Antagonistic activity of endophytic bacterial isolates against *S. rolfsii* in vitro

## Conclusion

The study identified effective nodule endophytic bacterial isolates against *S. rolfsii* under *in vitro* conditions. Further evaluation of these isolates for their plant growth promotion and under field conditions can fully revealed their potentiality for sustainable management of stem rot disease in groundnut crop. The study highlighted the potential of nodule endophytic bacteria as a sustainable and eco-friendly alternative method to chemical control in groundnut stem rot disease management.

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