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# Antagonistic Nodule Endophytic Bacteria Effective Against (*Sclerotium rolfsii*) Causing Stem Rot Disease in 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.*

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## 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 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. Eco-friendly alternatives such as the use of microbial antagonists including bacterial endophytes is an sustainable approach. In this context, the 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.

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Eight different isolates of *S. rolfsii* and twenty 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.*

## 1. 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 [1]. The production is estimated at 7.82 million tonnes with majority of the crop cultivated in the states of Gujarat, Rajasthan, Andhra Pradhesh, Tamil Nadu, Karnataka and Telangana (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 [2]. It is a ubiquitous, polyphagous soil-borne pathogen with an extensive host range, affecting over 500 different plant species and is [3] and is characterized by rapid mycelial growth and resilient sclerotia, which result in considerable economic losses [4]. 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-friendliy alternative to fungicides. The potential of rhizosphere microorganisms and endophytes has been reported in managing *S. rolfsii* [5,6]. 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. [7,8]. Nodules in legume plants such as groundnut contain diverse endophytic bacteria including rhizobial and non-rhizobial endophytes (NRE) [9]. 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.* [10]. Despite the potential of endophytes in stress management and adequate knowledge on the nodule endophytes [11] studies on bio-control potential of nodule endophytic bacteria against stem rot disease in groundnut crop are 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.

## 2. MATERIALS AND METHODS

### 2.1 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 rolfsii*) and endophytic bacteria respectively. 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 [12]. The samples were carried in ice box during transportation to the laboratory.

### 2.2 Isolation and Characterization of the Pathogen

The pathogen *S. rolsfii* 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 [13]. 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±2ºC. 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.

## 2.3 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 [5]. 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 [14]. Thirty day old seedlings were inoculated with 25 g 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.

## 2.4 Isolation and Characterization of Nodule Endophytic Bacteria

The endophytic bacteria from root nodules of groundnut were isolated based on the method given by [11]. The nodules were separated from the roots carefully avoiding any damage and were surface-sterilized using 3 per cent sodium hypochlorite for 30 s. 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 $10^{-12}$ 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.

## 2.5 *In vitro* Efficacy of Nodule Bacterial Endophytes Against *S. rolfsii*

The nodule endophytic bacteria were screened *in vitro* for their antagonism against the stem rot pathogen *S. rolfsii* by dual culture technique [15,5]. Loopful of 24h-old pure cultures of test isolate was streaked 1 cm away from the periphery of the PDA plates. Thereafter 5 mm mycelial disc of 5-day old culture of *S. rolfsii* was placed at the opposite end and the plates were incubated at 25±2°C. A control plate with only *S. rolfsii* 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 [16].

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

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).

## 3. 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). 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., [5] 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. rolfsii* resulted in higher disease incidence of 35 per cent in brinjal fields of Karnataka district. These differences might be due to the differences in the virulence of the pathogen across locations, edaphic factors, climate and agronomic practices [17].

### 3.1 Identification and Characterization of the Pathogen

The pathogen was identified as *S. rolfsii* based on the mycelial and sclerotial characters [18]. A total of 8 pure cultures *S. rolfsii* were obtained from the collected stem rot infected groundnut samples. All the isolates of *S. rolfsii* produced white cottony mycelial growth on PDA media characterized by profuse to highly profuse colony growth (Table 2).

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. [5] and Kumar et al., [19] who also reported on *S. rolfsii* 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 1.61 mm (GNSR 1). The average weight of 100 sclerotia ranged from 0.032 (GNSR 6) to 0.126 g (GNSR 5). Colour of the mycelium varied from light brown to dark brown (Table 3).

Variability among the pathogen isolates was reported by Le et al., [20] Thilagavathi et al., [21] Akram et al., [22] and more recently Akash et al., [5] 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 [17]. Sclerotial characteristics like density, viability, time taken for maturation are influenced by several soil type, soil pH etc. [23]. *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.

### 3.2 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 GNSR1) to 7 (isolate GNSR5) days and DPW ranged from 12.5 (isolate GNSR1) to 21.5(isolate GNSR7) days. The iolate GNS1 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.

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., [5] 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., [5] and the current study.

### 3.3 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 [24]. 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 [25]. It is also commonly employed to differentiate bacterial genotypes on culture plates and serves as a valuable marker of ecological diversity [26].

### 3.4 *In vitro* Efficacy of Nodule Endophytic Bacteria Against *S. rolfsii*

All the 20 endophytic bacterial isolates were tested for their antagonism against *S.rolfsii 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 [27]. 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. [28] isolated 16 bacterial endophytes from groundnut and reported isolate EGN1 as most potential against *S. rolfsii*. Akash et al. [5] identified most effective bacterial endophytes isolated from rhizosphere of groundnut as *Bacillus subtilis.* Maheswari et al. [29] 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. [30] isolated 45 bacterial strains from healthy peanut plants, with 6 showing antagonistic activity against *S. rolfsii*. Among these, *Bacillus* sp. Isolate F-1 and *Burkholderia* sp. Isolate R-11 exhibited the strongest activity, with inhibition zone widths of 20.25 mm and 15.49 mm, respectively. Koopa and Krishnaraj [31] found that the *Pseudomonas* isolate AUDP 48 exhibited the most significant

**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 crop (DAS) | PDI of *Sclerotium* stem rot (%) |
|---|---|---|---|---|---|---|
| **Mandal** | **Village** | **Latitude** | **Longitude** | | | |
| 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 |
| **Mean** | | | | | | **19.04** |

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

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

*#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*

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

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

*#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*

**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[a] | 12.50[a] |
| GNSR 2 | 5.50[d] | 15.25[b] |
| GNSR 3 | 5.00[c] | 15.00[b] |
| GNSR 4 | 5.50[d] | 14.75[b] |
| GNSR 5 | 7.00[e] | 18.50[d] |
| GNSR 6 | 5.50[d] | 17.00[c] |
| GNSR 7 | 5.00[c] | 21.50[e] |
| GNSR 8 | 4.75[b] | 15.25[b] |

*#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*

inhibition of *S. rolfsii* mycelial growth using the dual culture method. Similarly, Paramasivan et al., [32] reported six *Pseudomonas* sp. isolates tested for antagonistic activity against *S. rolfsii,* 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.

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

| Isolate | Shape | Margin | Elevation | Size | Texture | Appearance | Pigment | Gram staining reaction |
|---|---|---|---|---|---|---|---|---|
| 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 |

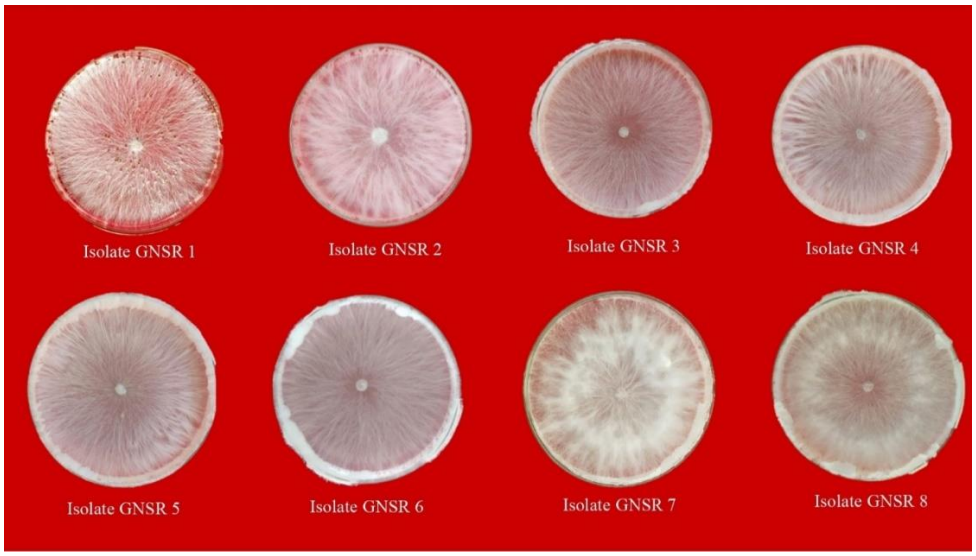


**Fig. 1. Isolates of pathogen *Sclerotium rolfsii***

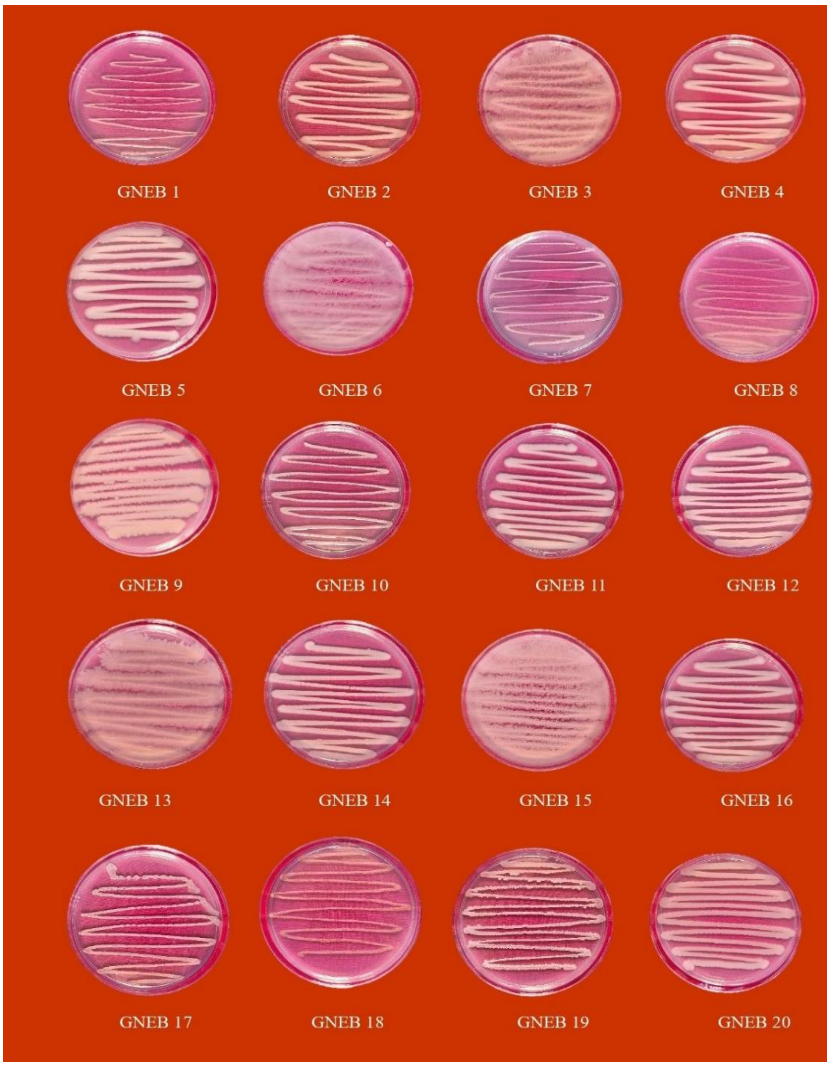


**Fig. 2. Pure cultures of endophytic bacterial isolates on nutrient agar media**

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

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

*#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*

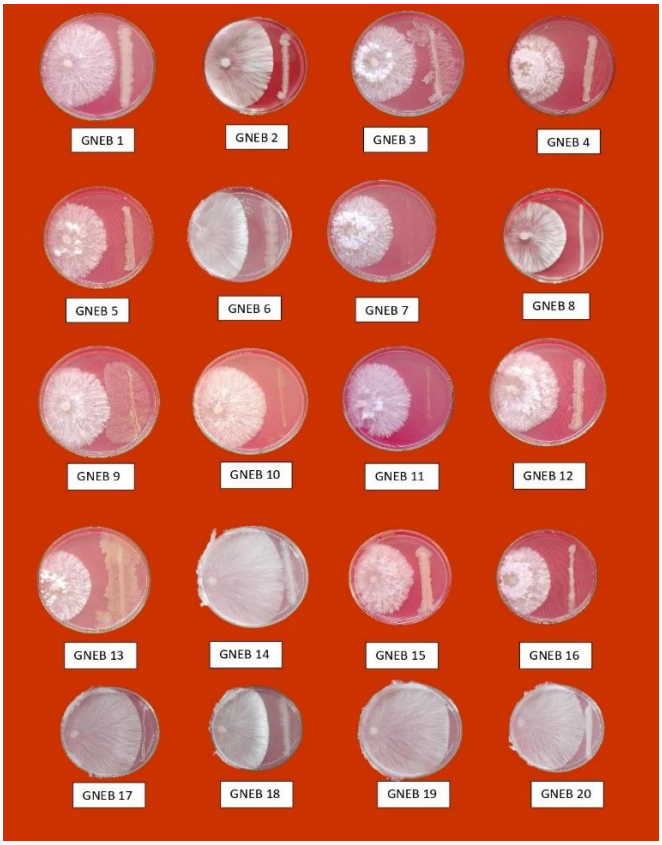


**Fig. 3. Antagonistic activity of endophytic bacterial isolates against *S. rolfsii In vitro***

In the present study, isolates of the stem rot pathogen *S. rolfsii* 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. rolfsii* isolate GNS1 by dual culture technique and two isolates GNEB13 and GNEB 6 were the most effective in inhibiting the mycelial growth of *S. rolfsii* isolate GNS1. These two isolates have the potential to be evaluated under field conditions for sustainable management of stem rot disease in groundnut.

## 4. 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.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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