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# Characterization of endophytic bacteria associated with sesame (*Sesamum indicum* L.) roots for plant growth-promoting potential

**J Ramya, M Gurivi Reddy , K Sakthivel, R Sarada Jaya Lakshmi Devi and D V Sai Ram Kumar**
Department of Plant Pathology, Acharya N G Ranga Agricultural University,
Agricultural College, Bapatla - 522101, Andhra Pradesh, India

## ABSTRACT

Sesame (*Sesamum indicum* L.) is an important oilseed crop, and the use of endophytic bacteria as plant growth-promoting microorganisms offers a sustainable approach to enhance its productivity. The present study was undertaken to isolate and evaluate endophytic bacterial isolates from sesame roots collected from different districts of Andhra Pradesh and Telangana. A total of 19 endophytic bacterial isolates were recovered and screened for plant growth-promoting traits, including phosphate, potassium and zinc solubilization, siderophore production, and hydrogen cyanide (HCN) production. Among the isolates, three exhibited phosphate-solubilizing activity, four showed potassium solubilization, fourteen demonstrated zinc solubilization, eight produced siderophores, and only two were positive for HCN production. Isolates SiRe-10, SiRe-11, SiRe-17, and SiRe-19 expressed multiple plant growth-promoting traits, indicating their potential as promising bioinoculants for improving nutrient availability and sesame productivity. These findings highlight the diversity of endophytic bacteria associated with sesame roots and their potential application in sustainable agriculture.

**Key Words**: *Bacillus spp, Endophytic bacteria, Nutrient solubilization, Plant growth promotion and Sesamum indicum*

Sesame (*Sesamum indicum* L.) is one of the oldest cultivated oilseed crops and is popularly known as the "Queen of Oilseeds" because of its superior oil quality and nutritional value (Kumar and Goel, 1994). It belongs to the family Pedaliaceae, order Tubiflorae, and possesses a chromosome number of 2n = 26 (Nayar, 1984). The genus *Sesamum* comprises about 37 species, among which *Sesamum indicum* L. is the most widely cultivated species distributed mainly in Africa, India, and the Far East (Kobayashi, 1991). Sesame is believed to have originated in Africa and is now extensively cultivated in tropical and subtropical regions for its edible seeds and high-quality oil.

Sesame seeds contain approximately 46 to 52 per cent edible oil rich in unsaturated fatty acids, particularly oleic acid (47%) and linoleic acid (39%), which contribute to lowering blood cholesterol levels (Shyu and Hwang, 2002). The seeds are also an excellent source of proteins, carbohydrates, vitamins, minerals and natural antioxidants such as sesamin, sesamoline, and sesamol that impart remarkable oxidative stability to sesame oil (Shyu and Hwang, 2002). Owing to its superior nutritional quality and medicinal significance, sesame is widely utilized in food, pharmaceutical, cosmetic and industrial sectors.

Globally, sesame cultivation has increased steadily due to the growing demand for healthy vegetable oils and plant-based protein sources. Sudan is the leading producer of sesame, followed by China, Myanmar and India (FAOSTAT, 2023). In India, sesame is cultivated over an area of about 15.23 lakh hectares with an average productivity of 527 kg $ha^{-1}$ during 2023–24. Major sesame-growing states include Gujarat, Rajasthan, Madhya Pradesh, Uttar Pradesh, West Bengal, Tamil Nadu and Andhra Pradesh (Vishwakarma *et al*., 2024).

Endophytes are endosymbiotic microorganisms, primarily bacteria and fungi, that colonize the intercellular and intracellular tissues of plants without causing any apparent disease symptoms (Pimentel *et al.,* 2011; Singh and Dubey, 2015). The term endophyte was first introduced by De Bary (1866), who defined endophytes as organisms that grow within plant tissues. These microorganisms are

ubiquitous in nature and establish complex relationships with their host plants, ranging from mutualism to antagonism and, rarely, parasitism (Nair and Padmavathy, 2014).

Endophytic microorganisms play a crucial role in plant growth and development by enhancing nutrient acquisition, promoting growth through phytohormone production, and improving plant tolerance to various biotic and abiotic stresses (Joseph and Priya, 2011; Parthasarathi *et al*., 2012). Many endophytic bacteria possess plant growth-promoting traits such as phosphate, potassium and zinc solubilization, siderophore production, biological nitrogen fixation, hydrogen cyanide production, and synthesis of phytohormones including indole-3-acetic acid (IAA). These characteristics enable endophytes to improve nutrient availability, suppress phytopathogens, and enhance overall plant health and productivity.

Among endophytic bacteria, species belonging to the genus *Bacillus* are of particular interest because of their ability to form endospores, survive under adverse environmental conditions, and produce a wide range of bioactive metabolites. Endophytic *Bacillus* spp. have been reported to enhance plant growth, improve nutrient uptake, induce systemic resistance, and confer tolerance against environmental stresses. Therefore, isolation and characterization of endophytic bacterial communities from sesame roots may provide potential candidates for the development of sustainable bioinoculants aimed at improving sesame productivity and soil health.

## MATERIAL AND METHODS

### Collection of Sesame Root Samples

Healthy sesame (*Sesamum indicum* L.) root samples were collected from different sesame-growing districts of Andhra Pradesh and Telangana for the isolation of endophytic bacteria. The samples were carefully uprooted, placed in sterile polythene bags, transported to the laboratory, and processed immediately for isolation studies.

### Isolation of Endophytic Bacterial Isolates from Sesame Roots

Isolation of endophytic bacterial isolates was carried out following a protocol given by (Gyaneshwar *et al*.,2001), root samples were washed thoroughly under running tap water to remove adhering soil particles and debris, followed by rinsing with sterile distilled water. The roots were cut into small segments and surface sterilized using 70 per cent ethanol for 2 to 3 min followed by 1 per cent sodium hypochlorite solution for 2 to 3 min. The sterilized root segments were rinsed three times with sterile distilled water to remove traces of sterilizing agents and dried aseptically. The surface-sterilized tissues were macerated in sterile distilled water using a sterile mortar and pestle to obtain a homogeneous suspension. For selective isolation of spore-forming *Bacillus* spp., the suspension was subjected to heat treatment at 80 °C for 10 min. Serial dilutions were prepared and aliquots were spread onto Nutrient Agar (NA) plates. The plates were incubated at 28 ± 2 °C for 24 to 48 h. Colonies exhibiting distinct bacterial morphology were selected and purified by repeated streaking on fresh NA medium. Purified isolates were maintained on nutrient agar slants at 4 °C for further studies.

### Screening of Endophytic Bacterial Isolates for Plant Growth-Promoting Traits

### Phosphate Solubilization

Phosphate-solubilizing ability of the endophytic bacterial isolates was assessed on Pikovskaya's agar medium. The medium contained (per litre): yeast extract, 0.5 g; dextrose, 10 g; tricalcium phosphate [$Ca_3 (PO_4)_2$], 5 g; ammonium sulphate [$(NH_4)_2 SO_4$], 0.5 g; potassium chloride 0.2 g; magnesium sulphate ($MgSO_4 \cdot 7H_2O$), 0.1 g; manganese sulphate ($MnSO_4 \cdot H_2O$), 0.0001 g; ferrous sulphate ($FeSO_4 \cdot 7 H_2O$), 0.0001 g; and agar, 15 g. The bacterial isolates were streaked onto the medium and incubated at 28 ± 2 °C for 72 h. Formation of a clear halo zone around the colonies was considered positive for phosphate solubilization (Katznelson and Bose, 1959).

### Potassium Solubilization

Potassium solubilization by the endophytic bacterial isolates was determined following the method of Aleksandrov *et al* (1967). The isolates were spot inoculated on Aleksandrov agar medium containing glucose, 5 g; $MgSO_4 \cdot 7H_2O$, 0.005 g; $FeCl_3$ , 0.1 g; $CaCO_3$ , 2 g; $Ca_3 (PO_4)_2$, 2 g; mica powder, 3 g; agar, 20 g; and distilled water, 1000 ml (pH 7.2). The inoculated plates were incubated at 37 °C for 3 to 5 days. Development of a clear halo zone around the colonies was recorded as positive for potassium solubilization

**Zinc Solubilization**

The zinc-solubilizing ability of the bacterial isolates was evaluated using Tris-mineral agar medium as described by Gandhi and Muralidharan (2016). The medium contained (per litre): D-glucose, 10 g; $(NH_2 SO_4)_2$ ,1.0 g; KCl, 0.2 g; $K_2 HPO_4$ , 0.1 g; and $MgSO_4$ , 0.2 g (pH 7.0). A loopful of overnight-grown bacterial culture was inoculated onto the medium and incubated at 29 ± 2 °C for 10 days. Zinc-solubilizing isolates were identified by the formation of a clear halo zone around the colonies.

**Siderophore Production**

Qualitative siderophore production was assessed using Chrome Azurol S (CAS) agar medium following the method of Schwyn and Neilands (1987). The bacterial isolates were spot inoculated on CAS agar plates and incubated at 28 ± 2 °C. Formation of an orange halo zone around the colonies on the blue CAS agar medium indicated siderophore production (Louden *et al*., 2011).

**Hydrogen Cyanide (HCN) Production**

Hydrogen cyanide production was evaluated following the method of Schippers and Bakker (1990). Twenty-four-hour-old bacterial cultures were streaked onto nutrient agar amended with glycine. A Whatman No. 1 filter paper soaked in 0.5 per cent picric acid prepared in 2 per cent sodium carbonate solution was placed inside the lid of each Petri plate. The plates were sealed with parafilm and incubated at 28 ± 2 °C for four days. A change in the colour of the filter paper from yellow to light brown or dark brown was considered positive for HCN production.

## RESULTS AND DISCUSSION

**Collection of Sesame Root Samples**

A total of 19 endophytic bacterial isolates were recovered from the root tissues of sesame from Srikakulam, Vizianagaram, and Kadapa districts of Andhra Pradesh and Warangal, Nalgonda and Mahbubnagar districts of Telangana (Table 1)

**Isolation of Endophytic Bacterial Isolates from Sesame Roots**

A total of 19 endophytic bacterial isolates were recovered from the root tissues of sesame following surface sterilization and culturing on nutrient agar medium. The isolates exhibited considerable variation in colony morphology, including differences in colony size, shape, colour, margin, elevation and texture. The purified isolates were maintained on nutrient agar slants and subsequently used for characterization and evaluation of plant growth-promoting traits.A representative purified isolate was shown in plate 1.

**Table 1. List of Endophytic bacterial isolates isolated from the sesame roots**

| S. No | Isolate name | District | Village | Source of Isolation |
|---|---|---|---|---|
| 1 | SiRe-1 | Srikakulam | Ampolu | Healthy sesame roots |
| 2 | SiRe-2 | | Kothavalasa | |
| 3 | SiRe-3 | | Denkada | |
| 4 | SiRe-4 | Vizianagaram | B. Rajeru | |
| 5 | SiRe-5 | | Kompangi | |
| 6 | SiRe-6 | | Gantyada | |
| 7 | SiRe-7 | Kadapa | Duvuru | |
| 8 | SiRe-8 | | Kothapeta | |
| 9 | SiRe-9 | | Moragudi | |
| 10 | SiRe-10 | | Yerraguntla | |
| 11 | SiRe-11 | Warangal | Jagtial | |
| 12 | SiRe-12 | | Kalleda | |
| 13 | SiRe-13 | | Nekkonda | |
| 14 | SiRe-14 | Nalgonda | Nakerekal | |
| 15 | SiRe-15 | | Panikera | |
| 16 | SiRe-16 | | Kampasagar | |
| 17 | SiRe-17 | Mahbubnagar | Narayanpet | |
| 18 | SiRe-18 | | Jadcherla | |
| 19 | SiRe-19 | | Kotakonda | |



**Plate 1. Bacterial isolate**

## Screening of Endophytic Bacterial Isolates for Plant Growth-Promoting Trait

### Phosphate Solubilization

A total of 19 endophytic bacterial isolates were evaluated for their ability to solubilize phosphate using an appropriate agar medium. Out of these, three isolates exhibited positive phosphate-solubilizing activity, while the remaining 16 isolates did not show any visible halo zone formation and were considered negative. Among the positive isolates, SiRe-10 showed high phosphate-solubilizing activity (++), whereas SiRe-9 and SiRe-11 exhibited moderate phosphate-solubilizing activity (+). The remaining isolates, namely SiRe-1, SiRe-2, SiRe-3, SiRe-4, SiRe-5, SiRe-6, SiRe-7, SiRe-8, SiRe-12, SiRe-13, SiRe-14, SiRe-15, SiRe-16, SiRe-17, SiRe-18 and SiRe-19, did not exhibit phosphate-solubilizing activity and were therefore classified as negative (Table 2 and Plate 2).

### Potassium Solubilization

A total of 19 endophytic bacterial isolates were evaluated for their ability to solubilize potassium using an appropriate agar medium. Among them, only four isolates exhibited positive potassium-solubilizing activity, while the remaining fifteen isolates did not produce any visible halo zone and were considered negative (High potassium solubilization (++) was observed in isolates SiRe-3 and SiRe-10, indicating their higher efficiency in mobilizing insoluble potassium

**Table 2. Characterization of endophytic bacterial isolates for plant growth promoting activities**

| S. No | Isolate name | Zinc solubilization | Phosphate solubilization | Potassium solubilization | HCN | Siderophore |
|---|---|---|---|---|---|---|
| 1 | SiRe-1 | + | - | - | - | ++ |
| 2 | SiRe-2 | - | - | - | - | + |
| 3 | SiRe-3 | - | - | ++ | - | ++ |
| 4 | SiRe-4 | + | - | - | - | + |
| 5 | SiRe-5 | - | - | - | - | ++ |
| 6 | SiRe-6 | + | - | - | - | + |
| 7 | SiRe-7 | - | - | - | - | - |
| 8 | SiRe-8 | - | - | - | - | + |
| 9 | SiRe-9 | + | + | - | - | + |
| 10 | SiRe-10 | + | ++ | ++ | - | - |
| 11 | SiRe-11 | ++ | + | + | - | - |
| 12 | SiRe-12 | + | - | - | - | - |
| 13 | SiRe-13 | + | - | - | - | - |
| 14 | SiRe-14 | ++ | - | - | - | - |
| 15 | SiRe-15 | + | - | - | - | - |
| 16 | SiRe-16 | + | - | - | - | - |
| 17 | SiRe-17 | ++ | - | + | + | - |
| 18 | SiRe-18 | + | - | - | - | - |
| 19 | SiRe-19 | +++ | - | - | + | - |

+++: Extremely positive, ++: Highly positive, +: Moderately positive, -: Negative



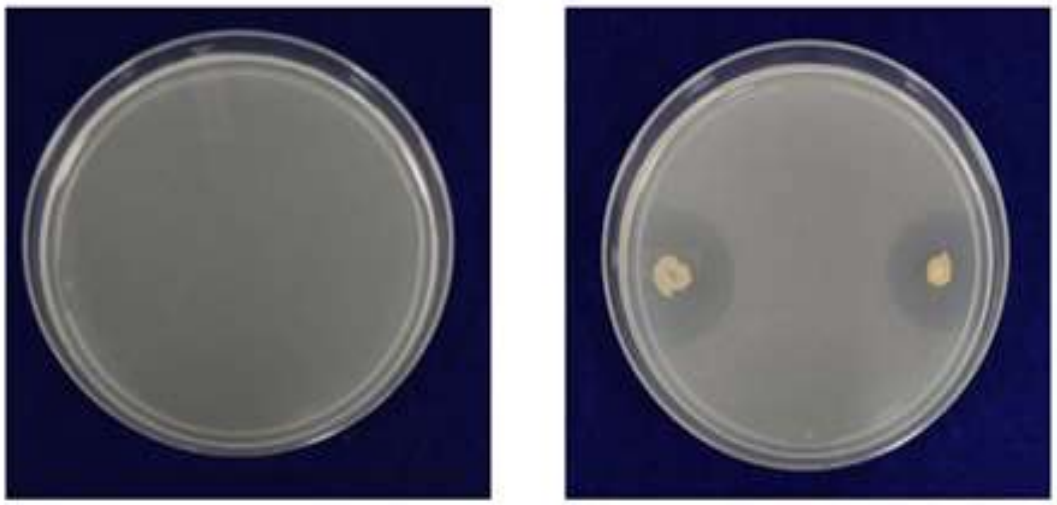
**Control** **SiRe - 10**

**Plate 2. Phosphate solubilization**

sources. Moderate potassium-solubilising activity (+) was recorded in isolates SiRe-11 and SiRe-17. The remaining isolates, namely SiRe-1, SiRe-2, SiRe-4, SiRe-5, SiRe-6, SiRe-7, SiRe-8, SiRe-9, SiRe-12, SiRe-13, SiRe-14, SiRe-15, SiRe-16, SiRe-18 and SiRe-19, did not exhibit potassium solubilization and were classified as negative (Table 2 and Plate3).

**Zinc solubilization**

A total of 19 endophytic bacterial isolates were screened for their zinc-solubilizing ability using zinc solubilization agar medium. Among the isolates tested, 14 isolates exhibited positive zinc solubilization as evidenced by the formation of a clear halo zone around the colonies, whereas five isolates (SiRe-2, SiRe-3, SiRe-5, SiRe-7 and SiRe-8) did not produce any halo zone and were categorized as negative. Considerable variation in zinc-solubilizing efficiency was observed among the positive isolates. One isolate, SiRe-19, exhibited extremely high zinc-solubilizing activity (+++). Three isolates, namely SiRe-11, SiRe-14 and SiRe-17, showed high zinc-solubilizing activity (++). The remaining ten positive isolates (SiRe-1, SiRe-4, SiRe-6, SiRe-9, SiRe-10, SiRe-12, SiRe-13, SiRe-15, SiRe-16 and SiRe-18) exhibited moderate zinc-solubilizing activity (+). The results indicate substantial variability among the endophytic bacterial isolates with respect to zinc solu-



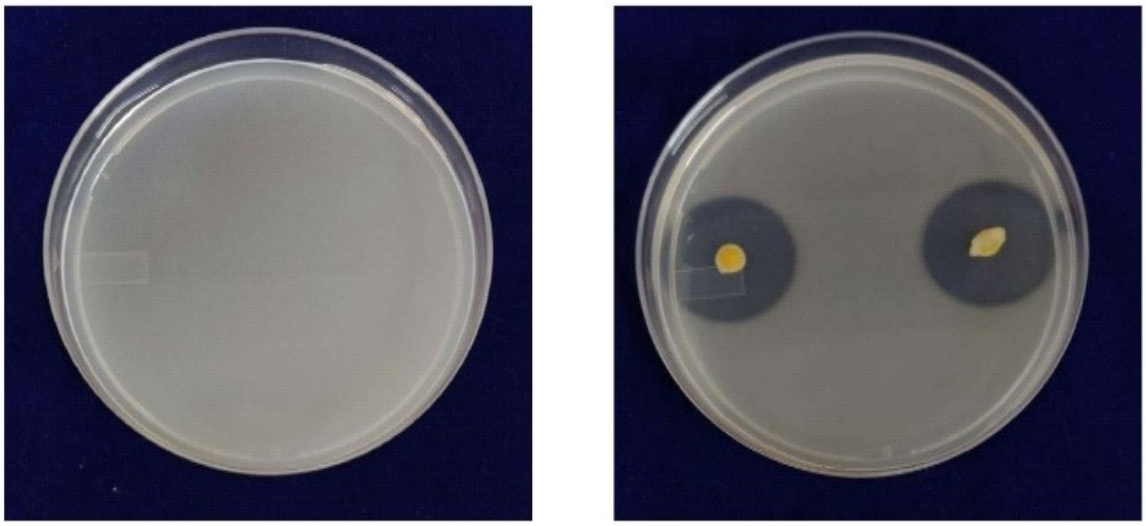
**Control** **SiRe - 10**

**Plate 3. Potassium solubilization**

bilization, suggesting their potential role in improving zinc availability in soil and promoting plant growth (Table 2 and Plate4).

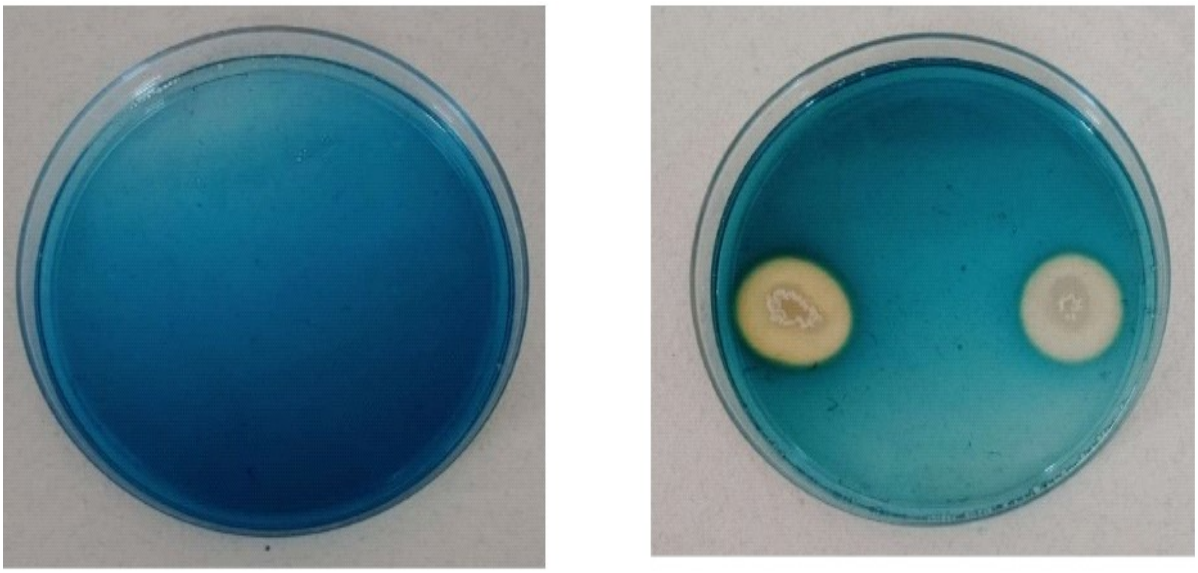
**Siderophore Production**

A total of 19 endophytic bacterial isolates were evaluated for siderophore production using the CAS assay (Table 2 and Plate5). Of these, eight isolates found positive siderophore production, while the remaining eleven isolates were negative. Among the positive isolates, SiRe-1, SiRe-3 and SiRe-5 exhibited high siderophore production (++), whereas SiRe-2, SiRe-4, SiRe-6, SiRe-8 and SiRe-9 showed moderate siderophore production (+), indicating variability in iron-chelating ability among the isolates. The remaining isolates, namely SiRe-7, SiRe-10, SiRe-11, SiRe-12, SiRe-13, SiRe-14, SiRe-15, SiRe-16, SiRe-17, SiRe-18 and SiRe-19, did not exhibit



**Control** **SiRe - 14**

**Plate 4. Zinc solubilization**

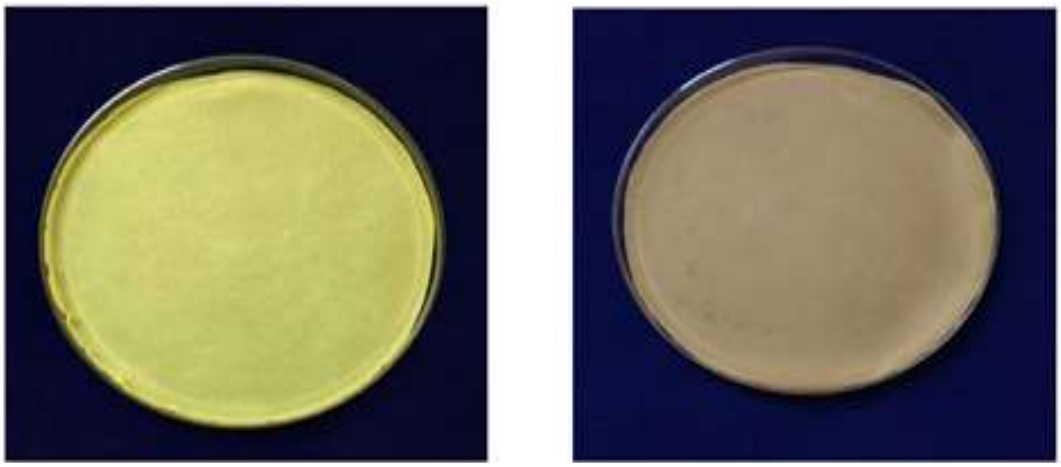


**Control** **SiRe - 1**

**Plate 5. Siderophore production**

**HCN Production**

Out of the 19 bacterial isolates evaluated for hydrogen cyanide (HCN) production, only two isolates, namely SiRe-17 and SiRe-19, exhibited a positive reaction, while the remaining seventeen isolates were negative. The positive isolates were confirmed by the characteristic colour change of picric acid–impregnated filter paper from yellow to brown following incubation on glycine-amended nutrient agar (Table 2 and plate 6).



**Control** **SiRe - 19**

**Plate 6. HCN Production**

## CONCLUSION

The present study demonstrated the occurrence of diverse plant growth-promoting (PGP) traits among sesame root-associated endophytic bacterial isolates. Of the 19 isolates evaluated, three exhibited phosphate solubilization, four potassium solubilization, fourteen zinc solubilization, eight siderophore production and two HCN production. The expression of multiple PGP traits by isolates SiRe-10, SiRe-11, SiRe-17 and SiRe-19 indicates their potential as promising bioinoculants for sustainable sesame production.

The phosphate-solubilizing activity observed in the present study is consistent with the findings of Joshi *et al*. (2023), who reported phosphate-solubilizing bacteria from the rhizosphere of *Dalbergia sissoo* with multiple plant growth-promoting attributes. Similarly, Nithyapriya *et al*. (2021) highlighted the role of endophytic bacteria in phosphate solubilization and other growth-promoting mechanisms. The predominance of zinc-solubilizing isolates corroborates the reports of Yadav *et al*. (2023) and Chaudhary *et al*. (2023), who demonstrated efficient zinc solubilization by rhizospheric and endophytic bacteria, respectively. The occurrence of siderophore and HCN production further emphasizes the multifunctional nature of the isolates. Overall, the present findings suggest that sesame-associated endophytic bacteria possess significant potential for enhancing nutrient availability and can be exploited as effective bioinoculants for sustainable sesame cultivation

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