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Original article

Enhancing sunflower growth and defense against *Sclerotinia sclerotiorum* stress with cyanogenic bacterial endophytes

Rajwinder Kaur[1], Pratibha Vyas[2*], Sandeep Sharma[3], Pankaj Sharma[4]

[1] Department of Microbiology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, India

[2] Department of Microbiology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, India

[3] Department of Soil Science, College of Agriculture, Punjab Agricultural University, Ludhiana, India

[4] Oilseed Section, Department of Plant Breeding and Genetics, College of Agriculture, Punjab Agricultural University, Ludhiana, India

*Corresponding address: vyasp2000@pau.edu

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Abstract

The present study was designed to check the ability of cyanogenic antagonistic bacterial endophytes to produce volatile organic compounds and enhance growth of sunflower under *Sclerotinia sclerotiorum* stress. Forty endophytic bacteria were isolated and screened for antagonism against *Sclerotinia sclerotiorum*. Out of 40 endophytes, two isolates SEB_1 and SEB_{21} exhibited fungal growth inhibition of 30.0 and 36.6%, respectively, in dual plate assay and 78.7 and 82%, respectively, in liquid media. Scanning electron microscopy showed rupturing, breakage and shrinkage of the fungal hyphae in the presence of the antagonistic bacterial strains. In addition, the bacterial strains also exhibited phosphate solubilization, and production of hydrogen cyanide, siderophores, auxins, ammonia, lipase and cellulases. The isolates were identified as *Sphingobacterium solani* SEB_1 and *Pseudomonas* sp. SEB_{21} by 16S rRNA gene sequencing. GCMS analysis confirmed the presence of dimethyl-sulfone, phenol, 3-hexadecene, 2,4-ditertbutylphenol, 1-docosene, 1-octadecene, 2,4-dimethylphenylimino-4-nitrophenyl, nonylcyclopropane 1-nonadecene, octacosane, n-octadecanol, isopropyl myristate, 3-hexadecene, 7,9-di-tert-butyl-1-oxosiro-4,5-deca-6,9-diene, 3,5-bis (1,1-dimethylethyl)-phenol, isopropyl myristate, 7,9-ditert-butyl-1-oxospiro (4,5) deca-6,9-diene-2,8-dione, 1-tricosene and octacosanol as major volatile organic compounds produced by the bacterial strains. SEM analysis showed colonization of plant tissues by both the bacterial strains validating their endophytic nature. The bacterial treatments showed disease control up to 88.9% and significantly improved vigor index, plant growth, total phenol and chlorophyll content over the uninoculated control in sunflower grown under greenhouse conditions. The treatment *Ss*SEB_1+*P*s$pSEB_{21}$ in the presence of the fungal pathogen showed 1.41%, 0.47% and 6.8% N, P and K content in plants, respectively, which was the highest among all the treatments. Further field-level evaluations are recommended to validate the effectiveness of *Sphingobacterium solani* SEB_1 and *Pseudomonas* sp. SEB_{21} as biological control agents. *Sphingobacterium solani* as a biocontrol agent and a plant growth-promoting endophyte is reported here for the first time.

Key words: cyanogenic bacteria, fungal growth, greenhouse, *Sclerotinia sclerotiorum*, SEM, volatile organic compounds

Introduction

Sunflower (*Helianthus annuus* L.), is an herbaceous oilseed crop belonging to family Asteraceae and native to North and South America. It is one of the widely cultivated oilseed crops in different countries after soyabean and rapeseed (Pilorgé 2020). In India, Karnataka dominantly contributes in the production of sunflower (Rai *et al*. 2016). Sunflower oil is rich in polyunsaturated acids (30%) such as linoleic acid and oleic acid. Different parts of sunflower are used as anti-inflammatory, antipyretic, astringents, antihypertensive and stimulant agents as they synthesize approximately 28-102 bioactive compounds such as tocopherol and flavonoids including apigenin, quercetin, heliannone and kaempferol (Nguyen *et al*. 2021). It is a fast-growing crop with a deep root system and large biomass, which makes it an excellent phytoremediator, with the ability to eliminate harmful heavy metals from water and soil (Kara *et al*. 2013; Martins *et al*. 2014). Sunflower oil is used as a stabilizer, softening agent and antioxidant in pharmaceutical preparation (Aulton and Taylor 2013).

Sunflower is susceptible to various bacterial and fungal pathogens. Bacterial wilt, apical chlorosis, crown gall, stalk rot, alternaria blight, verticillium wilt and Southern blight are various diseases of sunflower caused by *Pseudomonas solanacearum, P. syringae*, *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Alternaria helianthin*, *Verticillum dahliae* and *Sclerotinia rolfsii*, respectively (Harveson *et al*. 2016). Fungal pathogens are the prime agents causing huge losses in yield and quality of the crop every year. Charcoal head rot, downy mildew, *Fusarium* stalk rot, *Botrytis* head rot, white rust, yellow rust, *Sclerotinia* stem and head rot and *Verticillum* wilt are various fungal diseases of sunflower (Gulya *et al*. 1991).

Sclerotinia sclerotiorum is one of the most disastrous soil-borne fungal pathogens causing huge losses in crops every year. Globally, it infects more than 408 plant species (Derbyshire *et al*. 2022). *Sclerotinia sclerotiorum* infection was reported for the first time in sunflower in 1861. It is a necrotrophic pathogen causing stem rot disease in sunflower and leads to stem breakage in plants. Under humid conditions, its spores germinate in plant hosts or it enters through the epidermis of a plant and leads to wilting or necrosis of plant tissues. Oxalic acid production is an important virulence factor of *Sclerotinia*, as oxalic acid leads to inhibition of callose deposition, oxidative burst and acidic conditions which enhance the activity of fungal enzymes in host plants (Wang 2024). It is most difficult to eradicate from fields because it can survive for years due

to its long-term survival structures called sclerotia and lack of appropriate methods of soil fumigation (Smolinska and Kowalska 2018).

Chemical fungicides including Triadimefon, Flusilazole and CaprioF-500 are used to control this fungal pathogen. However, the intensive use of these fungicides has serious impacts on the environment, and non-target microorganisms and other soil fauna. Additionally, chemical fungicides also cause resistance in pathogens and make them adaptable to adverse biotic and abiotic stresses (Goswami *et al*. 2018). Therefore, looking into the deleterious effects of chemical fungicides, it becomes important to find effective and eco-friendly alternatives to control the growth of the phytopathogens as a means of plant disease management. Biological control is an important and emerging method to suppress pathogens and various abiotic stresses in plants by using different living organisms or their extracts in sustainable ways (Kaur *et al*. 2018; Rabuske *et al* 2023). *Trichoderma harzianum* antagonizes the growth of *Sclerotinia sclerotiorum* by different mechanisms such as mycoparasitism, competition and production of cell wall degrading enzymes. *Trichoderma viride* bio-fungicide is commercially available for control of *Sclerotinia* (Topolovec-Pintarić *et al*. 2025). Endophytes are also effective biocontrol agents as these elicit plant defense through direct and indirect interaction with plant hormones such as the ability to produce various bioactive compounds, for example volatile organic compounds (VOCs), hydrolytic enzymes and hydrogen cyanide (Vyas and Singh 2024; Wielkopolan *et al*. 2025). The VOCs produced by endophytic bacteria serve as novel compounds with the ability to inhibit the growth of phytopathogens (Lopez *et al*. 2021). These compounds include alkanes, alkynes, esters, alcohols, cyanides, ketones, terpenoids, aldehydes, and sulphur-containing compounds. The endophytes producing these VOCs benefit plants by enhancing immunity against various phytopathogens, thereby, promoting plant growth and development (Vyas and Singh 2024). However, no reports are accessible on the effect of bacterial endophytes against stem rot disease of sunflower. Therefore, the present study was designed to isolate and evaluate bacterial endophytes as potential biocontrol agents against *Sclerotinia sclerotiorum,* production of volatile organic compounds, hydrolytic enzymes, and plant growth-promoting traits. The potential endophytes were further tested to enhance growth and nutrient content of sunflower under the stress of *Sclerotinia sclerotiorum* causing stem rot disease in sunflower.

Materials and Methods

Isolation and purification of bacterial endophytes

Healthy and disease-free sunflower plants grown in the fields of the University (30°56' N, 75°52'E, altitude 247m) were uprooted carefully and brought to the laboratory. Endophytic bacteria were isolated by surface sterilizing the roots, stems and leaves of sunflower by dipping in 0.2% mercuric chloride for three minutes, ethanol for two minutes and washing five times in sterile distilled water. Segments, 1-2 mm long, of tissues were placed on nutrient agar plates. The Petri plates were incubated at 28°C for 24-48 h (Tan *et al*. 2015; Kaur *et al.* 2025). Colonies growing in the vicinity of plant samples were purified on nutrient agar and morphologically distinct bacterial isolates were stocked in 30% glycerol (v/v) for further use.

Procurement and purification of *Sclerotinia sclerotiorum* culture

Sclerotinia sclerotiorum culture was acquired from the Department of Plant Pathology of the University. The fungus was sub-cultured on potato dextrose agar plates and preserved on slants for future use.

Screening the bacterial endophytes for antagonism against *S. sclerotiorum*

Dual plate assay was used to check the antagonistic potential of endophytic bacteria against *Sclerotinia sclerotiorum* under *in vitro* conditions. The plates in triplicates were incubated at 28˚C for five to six days. The inhibition percentage was calculated using the following formula:

$$\text{Reduction in fungal growth (\%)} = [(\text{Control-Treated})/\text{Control}] \times 100$$

Where *control* is the diameter of fungus in plates without any bacterial inoculation while *treated* is fungal diameter in plates with antagonistic bacteria.

Screening the antagonistic bacteria for the production of HCN, siderophores and hydrolytic enzymes

For the production of hydrogen cyanide, the bacterial antagonists were streaked on tryptone soya agar amended with glycine. A Whatman filter paper no. 1 was dipped into picric acid and placed on the inner side of the lid of each plate. Petri plates were sealed with parafilm, and kept at 28°C for 28-48 h for incubation. A change in color of the filter paper from yellow to brown indicates a positive result (Bakker and Schippers 1987).

Siderophore production was detected following the method of Schwyn and Neilands (1987). Briefly, pure cultures of bacteria were inoculated as spots on Chrome Azurol sulphonate agar and incubated at 30°C for five days. Orange zones around bacterial colonies were measured by subtracting the diameter of the bacterial colony from the total diameter of the zone including the diameter of the bacterial colony.

Cellulase production was detected by spot inoculating the pure bacterial cultures on minimal medium amended with carboxy methyl cellulose (Farkaš *et al*. 1985). After incubation, Petri plates were flooded with Congo red dye and zones of cellulose production were measured and the dissolution factor was determined as:

Dissolution Factor (DF)= (Zone diameter- colony diameter)/colony diameter.

Xylanase production was tested by spot inoculating the cultures on nutrient agar amended with xylan. The Petri plates were incubated at 28°C. After 48h incubation, the Petri plates were flooded with Congo red dye. The zones appearing around the bacterial cultures were measured (Farkaš *et al*. 1985).

Amylase production was tested by spot inoculating the cultures on starch agar medium plates and incubated at 30°C. After 48h, the plates were flooded with iodine solution and the clear zones around the bacterial colonies were measured (Claus 1989).

Proteolytic activity and pectin hydrolysis was detected by inoculating the bacterial cultures as spots on skim milk agar, and pectin agar, respectively. The Petri plates were incubated at 28°C and after 48h incubation, the clear zones which formed around the colonies were measured (Collmer *et al*. 1988; Claus 1989).

Lipolytic activity by the isolates was detected by spot inoculating the cultures on nutrient agar with 1% Tween-80. Petri plates were incubated at 28°C. After 48h incubation, the plates were flooded with methyl red indicator and clear halos around bacterial cultures indicated lipolytic activity of bacteria (Lusty and Doudoroff 1966).

Effect of antagonistic bacterial endophytes on the ultrastructure of *S. sclerotiorum*

For studying the effect of the bacterial endophytes on the structure of *S. sclerotiorum*, mycelium was collected from the edge of fungus growing near antagonistic bacteria from the dual plate assay. The fungal mycelium collected from plates without any bacterial inoculation served as the control. The fungal mycelium was fixed with 2.5%

solution of glutaraldehyde at 4°C and the solution was drained with 0.1 M solution of sodium cacodylate buffer three times at 15 min. intervals. Further, the sodium cacodylate buffer was drained off using osmium tetroxide and OsO_4 was drained off by washing with sodium cacodylate for 15 min. Samples were dehydrated with ethanol at different concentrations (30 to 100%) and dried in a laminar air flow cabinet. The samples were coated with gold particles according to standard protocol rules and morphological changes were studied at different magnifications (Kinden and Brown 1975).

Testing the compatibility between bacterial endophytes

The compatibility between the two cyanogenic endophytic bacterial antagonists was assessed by cross streak and dilution plate methods (Kannan and Sureender 2009; Sundaramoorthy *et al*. 2012). In the cross streak method, bacterial endophytes were streaked on the nutrient agar plates perpendicular to each other and the plates were incubated for 24-48h at 28°C. The plates were observed for growth on the junction of streaked lines or any inhibition among the bacterial endophytes.

For the dilution plate method, individual bacterial culture was grown separately into 10 ml nutrient broth (~ 10^8 CFU/ml) for 24-48h at 28°C. The bacterial cultures were diluted serially up to 10^{-7} and an equal volume of each culture (50 µl) from each dilution was put simultaneously on the center of nutrient agar plates, spread uniformly and the plates were incubated at 28°C for 24-48h. After incubation, the plates were observed to check whether bacterial cultures were compatible to each other or not. The simultaneous growth of both bacterial cultures in equal numbers indicated compatibility among bacterial isolates.

Effect of cyanogenic bacterial endophytes on the biomass of *S. sclerotiorum* in liquid medium

Two antagonistic bacterial isolates and their consortium were tested for their effect on the biomass of *S. sclerotiorum* in yeast malt extract broth. Flasks containing 100 ml broth were inoculated with a mycelia disc of 5 mm diameter along with 900 µl of 24 hour old bacterial culture. In dual bacterial inoculations, 450 µl of each culture was added along with the fungal culture. Control flasks without any bacterial endophytes were also maintained. The experiment had four treatments with three replicates: T1 Control (*S. sclerotiorum*); T2 SEB_1 +*S. sclerotiorum*; T3 SEB_{21}+*S. sclerotiorum* and

T4SEB$_1$+ SEB$_{21}$+*S. sclerotiorum*. The flasks were incubated in a rotatory shaker at 28°C. After five days of incubation, the contents were filtered with pre weighed filter paper and kept in a hot air oven for drying at 50°C. The weight of fungal mycelium was calculated by subtracting the weight of filter paper from the weight of the total content (filter paper + fungal mycelium).

Weight of fungus = Weight of Total content -Weight of filter paper.

Reduction in the biomass of fungus was studied by comparing bacterial inoculated flasks with the control.

Screening of potential antagonists for plant growth-promoting traits

Phosphate solubilization potential of bacterial cultures was screened by using modified Pikovaskaya agar with bromophenol dye (Gupta *et al*. 1994). Bacterial cultures were spot inoculated in the center of a plate and incubated at 28°C for five days. Clear zones around bacterial colonies were measured to determine solubilization of tricalcium phosphate by endophytic bacteria. The solubilization index was measured by subtracting the bacterial colony diameter from the total solubilization zone.

$$\text{Solubilization index (PSI)} = \frac{z+c}{c}$$

Where, z is the zone of solubilization and c is colony diameter.

Quantitative estimation of phosphate solubilization was done by using NBRIP (National Botanical Research Institute's Phosphate) broth, supplemented with 0.5% tricalcium phosphate. An aliquot of 50 µl of each bacterial culture was inoculated in 50 ml of NBRIP broth in triplicates and flasks were incubated in a shaking incubator for five days at 28°C. After incubation, the content was centrifuged for 10 min at 10,000 rpm. To estimate soluble phosphorus, 2.5 ml of Barton's reagent was added to 1 ml of supernatant and a final volume of 50 ml was made with distilled water in a volumetric flask. Absorbance of yellow color was taken on 430 nm after 10 minutes.

Ammonia production in bacteria was determined by using the Cappuccino and Sherman (1992) method. The cultures were inoculated in 10 ml peptone broth and were incubated at 28°C. After 48h, 0.5 ml Nessler's reagent was added to each tube including the control. A change from yellow to orange with the addition of Nessler's reagent indicates a positive result for ammonia production (Cappuccino and Sherman 1992).

Auxin production in bacteria was checked by using nutrient broth supplemented with 0.1% DL-tryptophan following Loper and Schroth (1986). A 50 µl bacterial culture was inoculated into tryptophan broth and tubes were incubated at 28°C for three to four days in the dark. After the incubation period, the culture was centrifuged at 10,000 rpm for 10-15 min. One milliliter supernatant was taken and 4 ml Salkowski reagent was added. The appearance of pink indicates positive results for auxin production. The absorbance was measured at 535 nm after 10 min.

Characterization of potential isolates by 16S rRNA gene sequencing

Following standard protocols 16S rRNA gene sequence analysis of the two potential strains was carried out (Gulati *et al*. 2008). The isolation of DNA was carried out using the Qiagen DNeasy Plant Mini Kit. The amplification of 16S rRNA gene, thermocycling conditions, and sequence analysis were done as described in detail earlier (Gulati *et al*. 2008). The universal primers fD1 (50 -AGAGTTTGA TCCTGGCTCAG-30) and rP2 (30 -ACGGCTACCTTGTT ACGACTT-50) were used for the amplification of 16S rDNA (Weisburg *et al*. 1991). BLASTN search program was used to compare sample sequences with GenBank database sequences. The sequence of the strains SEB1 and SEB21 and their related taxa were aligned with ClustalW and Kimura's two-parameter model using MEGA software package version 7 was used to find the evolutionary distance of the endophytic bacterial strains and their related taxa. The gene sequences of SEB1 and SEB21 were submitted to NCBI GenBank with the accession numbers PP061449 and PX484454, respectively.

Detection and identification of volatile organic compounds produced by endophytic bacterial antagonists

For the extraction of volatile organic compounds, bacterial isolates were streaked on nutrient agar media and 3 g activated charcoal was placed on the lids of Petri plates and sealed with parafilm. Plates were incubated at 28°C for five days in darkness. After incubation, charcoal was collected and washed with 5 ml of ethyl acetate, centrifuged three times and the supernatant was collected, and filtered with 0.45 µm micro syringe filters. One ml filtered supernatant was used to determine the volatile organic compounds with gas chromatograph (GC) coupled with mass spectrometer (MS).

For gas chromatograph, the oven temperature was programmed at 40°C. The temperature of the injector port was 250°C and the mode of injection was spit less. One

microliter sample was injected into the GC and the column flow rate was 1 milliliter per min. The peaks of VOCs were analyzed with mass-spectral analysis. Start m/z (mass/charge) was 40 and end m/z was 800 and event time was 0.50 seconds (Kai *et al*. 2020).

Validation of the endophytic nature of antagonistic bacterial endophytes

To validate the endophytic nature of bacterial strains, SEM (scanning electron microscopy) analysis was carried out. Sterilized sunflower seeds (20% in sodium hypochlorite for three min followed by washing three times with distilled water) were dipped in the two day old bacterial cultures for one hour and sown in sterilized vermiculite in plastic cups. The experiment had a total of three treatments with one uninoculated control and two bacterial treatments *Ss*SEB$_1$and *Pa*SEB$_{21}$.

Plants were uprooted after 10 days and roots along with stem sections were used for SEM analysis. Sections from plants without any bacterial inoculation were used as the control. Tissue sections from each treatment were fixed at 4°C in 2.5% glutaraldehyde, which was drained off after 24h with 0.1 M sodium cacodylate buffer three times at 15 min intervals. Sodium cacodylate was washed off using osmium tetroxide. Vials consisting of samples were incubated at 4°C for two to three h. Sodium cacodylate was used to drain OsO_4 for 15 min. Dehydration of the sample was done by using different concentrations of ethanol from 30 to 100%. Samples were coated using gold particles according to standard protocol and colonization of endophytes was observed at different magnifications by SEM.

Vigor index and root elongation bioassay

The antagonistic endophytic bacteria were inoculated in 100 ml nutrient broth and incubated at 28°C and 150 rpm for 48h. Sunflower seeds were surface sterilized by dipping in 20% sodium hypochlorite solution for three minutes, followed by washing with distilled water two to three times. Sterilized seeds were dipped into bacterial cultures for one hour and four seeds were placed per plate of 1% water agar. Seeds dipped into sterilized broth without any bacterial inoculation were taken as the control. The experiment was performed in triplicate with four treatments: T1 Control, T2 *Ss*SEB1, T3 *Pa*SEB$_{21}$, and T4 *Ss*SEB$_1$+*Pa*SEB$_{21}$. The Petri plates were incubated in the dark for five to seven days at 30°C. Germination percentage and radical length was

noted and vigor index was calculated. The following formula was used to calculate germination percentage:

Germination percentage = (Number of seeds germinated/Total no. of seeds) × 100

Vigor index was calculated using the following formula:

Vigor index = Germination (%) × Seedling length (cm)

Plant growth promotion and disease suppression in pots

The experiment was conducted in earthen pots with a capacity of holding 10 kg soil. Bacterial cultures were inoculated in nutrient broth and kept in a rotatory incubator for 48h at 28°C. For a single treatment, sterilized seeds of sunflower were dipped in 20 ml broth (~ 1×10^9 CFU/ml) for 30 minutes. In the combination of two bacteria, 10 ml broth of each culture was used. The study had a total of five treatments with three replicates: T1 Control, T2 *S. sclerotiorum,* T3 *Ss*SEB1+ *S. sclerotiorum*, T4 *Pa*SEB$_{21}$+*S. sclerotiorum*, and T5 *Ss*SEB$_1$+ *Pa*SEB$_{21}$+*S. sclerotiorum*. Seeds treated with bacterial culture were sown in pots, with three seeds per pot and kept in a greenhouse in the month of December. The plants were irrigated daily as per the requirement. During early flowering stage (approx. 50 days after sowing), plants were wounded or slashed using sterilized blades. Bits from seven day old *Sclerotinia sclerotiorum* growing on potato dextrose agar plates were inoculated on slashed parts of plants except in control T1. Fungal bits were secured by cotton and parafilm. The bacterial culture (~ 1×10^9 CFU/ml) was applied to the aerial part of the plants in the form of foliar spray immediately after the fungal inoculation. The plants were observed daily for the appearance of any disease symptoms. Plants were uprooted after 60 DAS and the growth parameters such as plant height (cm), root length (cm), shoot length (cm), fresh weight (g) and dry weight of plant (g), N, P and K content (%), chlorophyll content (mg/g), total phenolic compound (%) and disease incidences were noted.

Disease appearance symptoms such as yellowing and wilting in leaves, stem breakage and stem lesions in plants were observed and the disease index (%) was calculated according to the following equation:

Disease index (%) = (Total number of diseased plants/ Total no. of plants) × 100

Disease control (%) = [(100 (-) DI in treatment)/(Disease incidence in control)] × 100

Shoot samples of plants were dried in a hot air oven at 70°C and ground in a mortar and pestle to make a fine powder. Both soil and plant samples in powdered form were used to calcultate total nitrogen, phosphorus and potassium content. Phosphorous estimation was done according to the yellow color method (Jackson 1973). Available phosphorus in the soil was determined according to the sodium bicarbonate method (Olsen *et al*. 1954). Soil samples were kept overnight and electrical conductivity of soil was taken using solubridge or conductivity meter.

Total phenolic content was determined by following Swain and Hills (1959). Briefly, a 0.4 g dry and powdered shoot sample was taken and 5 ml of 80% methanol was added and kept for one hour. The content was filtered and the volume was adjusted to 10 ml with 80% methanol. Test tubes containing 0.5 ml methanol were kept in a hot water bath at 80˚C and 5-6 ml water were added to each test tube followed by the addition of 0.5 ml Folin-phenol reagent. One milliliter of sodium carbonate was added and absorbance of blue was noted on 760 nm.

Statistical analysis

A completely randomized design (CRD) was implemented for data analysis. The average value of triplicates was represented in the final data. Microsoft excel 2007 and SPSS (version 20.0) were used to analyze the results. The mean of the treatments was compared by post hoc Duncan or Tukey test at a confidence level of 95 percent.

Results

Screening of bacterial endophytes against *Sclerotinia sclerotiorum* by dual plate assay

Out of 40 isolates, five isolates (12.5%) showed antagonistic activity against *S. sclerotiorum* with 6.25, 10.0, 12.5, 30.0 and 36.6% growth inhibition of the fungal pathogen. The highest antagonistic activity exhibited by the bacterial endophyte SEB_1 (36.6%) followed by isolate SEB_{21} (30.0%) were selected for further studies (Fig. 1).

Effect on the ultrastructure of *S. sclerotiorum* by antagonistic bacterial endophytes

The effects of SEB1 and SEB21 on the fungal pathogen were examined by scanning electron microscopy. Changes in morphological features of fungal hyphae were observed in the presence of both antagonistic bacterial isolates SEB_1 and SEB_{21}. The scanning electron micrographs showed thinning and intense breakage of fungal hyphae

in the presence of $SEB_1$and SEB_{21} isolates (Fig. 2). The hyphae of *S. sclerotiorum* grown without inoculation of bacterial culture (control) were regularly shaped and healthy without any breakage or thinning.

Compatibility testing and effect of antagonistic bacteria on *S. sclerotiorum* in liquid medium

Bacterial cultures were checked for their compatibility by cross streaking methods and dilution plate assay. No inhibition zone was observed on the junction of streaked lines on cross-streaked plates. The bacterial isolates SEB1 and SEB21 showed 6.3×10^9 and 7.3×10^9 CFU/ml, respectively, on nutrient agar plates after 48h incubation, thus signifying that the bacterial isolates were compatible with each other.

Effect of cyanogenic bacterial endophytes on the biomass of *S. sclerotiorum* in liquid medium

Inhibition of fungal biomass in liquid medium by antagonistic bacteria in single inoculation and combinations varied from 78.7 to 88.1% (Table 1). The highest reduction in the fungal growth was observed by a combination of both bacterial isolates (SEB_1+SEB_{21}) with 88.1% inhibition followed by SEB_{21} (82.0%) and SEB_1 (78.7%). The bacterial treatments differed significantly from one another in inhibiting the growth of *Sclerotinia sclerotiorum*.

Screening for hydrolytic enzyme production and plant growth-promoting attributes

The antagonistic bacterial endophytes SEB_1 and SEB_{21} were tested for the production of hydrolytic enzymes cellulase, xylanase, amylase, protease, pectinase and lipase. Bacterial isolate SEB_{21} showed positive results for cellulase production, with a 7.0 mm zone on CMC agar plates. Lipase production was exhibited by both isolates with 13.0 and 11.1 mm clearance zone shown by SEB_1 and SEB_{21}, respectively. Both isolates tested negative for xylanase, amylase, protease and pectinase production.

In addition, both isolates tested positive for hydrogen cyanide production. The bacterial isolates SEB_1 and SEB_{21}exhibited 27.0 and 19.3-mm zones of siderophore production, respectively, on Chrome Azurol Sulphonate agar. The isolates were also able to produce ammonia as the color of peptone broth changed to orange for the isolate SEB_1 and orange-brown in the case of SEB_{21}on addition of Nessler's reagent. Both isolates were also able to produce auxins with 8.2 and 14.03 µg/ml by the isolate SEB_1

and SEB_{21}, respectively, in tryptophan-amended nutrient broth. The isolates SEB_1 and SEB_{21} showed phosphate solubilization indexes of 1.5 and 3.26, respectively, on modified Pikovskaya agar plates. The tricalcium phosphate solubilization exhibited by SEB_1 and SEB_{21} was 66.9 and 90.4 µg/ml, respectively, in NBRIP brth after five days of incubation.

Characterization and identification of antagonistic bacterial endophytes

On the basis of 16S rRNA gene sequencing, 1425 bp sequence of the isolate SEB_1 exhibited 99.3% similarity with *Sphingobacterium solani* strain MLS-26-JM13-11 and 1446 bp sequence of the isolate SEB_{21} showed 99.6% homology with *Pseudomonas* sp. strain LZU-2 (Fig. 3). The phylogenetic tree created by using 16S rRNA gene sequences of SEB_1, SEB_{21} and their nearest neighbors showed two different groups with the first group containing SEB_1 with strains of *Sphingobacterium* spp., *S. griseoflavum*, *S. solani*, and *S. bambusae.* The second group contained SEB21 with *Pseudomonas* sp*., P. stutzeri* and *P. putida* strains (Fig. 3).

Identification of volatile organic compounds (VOCs) produced by antagonistic bacterial endophytes

Bacterial antagonistic endophytes exhibited the production of various VOCs as detected by GCMS. The results revealed that the number of VOCs produced by *Sphingobacterium spritivorum* SEB_1 were more than *Pseudomonas* sp. SEB_{21} (Fig. 4). The volatile organic compounds produced by endophyte *Sphingobacterium solani* SEB_1 included methylsufinylmethane, dimethyl-sulfone, phenol, 2-hydroxybenzaldehyde, nonylcyclopropane, 3-hexadecene, 2,4-ditertbutylphenol, 1-docoesne, isopropyl myristate, 1,2-benzene-dicarboxylic acid, 7,9-di-tert-butyl-1-oxosiro-4,5-deca-6,9-diene, hexadecanoic acid, 1-octadecene, 2,6,10,15-tetramethylheptadecane, 2,4-dihydroxyphenyl, tetratriacontane, 2,4-dimethylphenylimino-4-nitrophenyl,1-nonadecene, octacosane, n-octadecanol, 7-methylhexadecane and tritetracontane (Fig. 4a). *Pseudomonas* sp. SEB_{21}were found to produce 3-tertradecane, 3-hexadecene 3,5-bis(1,1-dimethylethyl)-phenol, 3-hexadecene, 1-octadecene, isopropyl myristate, 7,9-ditert-butyl-1-oxospiro (4,5) deca-6,9-diene-2,8-dione, 1-tricosene and n-octadecanol. The compounds isopropyl myristate, 3-hexadecene, 1-octadecene, 7,9-di-tert-butyl-1-oxosiro-4,5-deca-6,9-dieneand n-octadecanol were the common VOCs produced by both endophytic strains (Fig. 4).

Validation of the endophytic nature of antagonistic bacterial endophytes

Antagonistic bacterial endophytes were analyzed by SEM studies to visualize bacterial endophytes in the plant tissues. Slightly curved and straight rods singularly and in chains were observed in the plant samples bacterized with the bacterial strains (Fig. 5). However, no bacterial colonization was observed in the samples of uninoculated plants. Colonization of bacterial endophytes inside plant tissue indicates that bacterial strains are of an endophytic nature.

Vigor index and root elongation bioassay

Two promising endophytic bacterial strains and their combination were tested for their ability to improve the vigor index of sunflower on 1% water agar. Seed germination was observed to vary from 50.0 to 75% by different treatments (Fig. 6; Table 2). The vigor index varied from 457.5 to 510.0% by bacterial treatments with respect to uninoculated control treatment (Table 2). The treatments differed significantly from one another in improving the seed vigor, with the highest vigor index shown by the treatment *Ss*SEB$_1$+*Pa*SEB$_{21}$ (Table 2). Minimum vigor index was observed in the treatment *Pa*SEB$_{21}$ (405.0%) among the bacterial treatments.

Disease suppression and plant growth enhancement by bacterial endophytes in pots under greenhouse conditions

The effect of *Sphingobacterium solani* SEB$_1$ and *Pseudomonas* sp. SEB$_{11}$ and their combination on plant growth, nutrient content and disease suppression was studied in pots under the stress of *Sclerotinia sclerotiorum*. The bacterial treatments showed an increase in root length of up to 20.2% over the uninoculated control and up to 60.2% over the *S. sclerotiorum* treatment (Fig. 7; Table 3). The highest increase was exhibited by the consortium treatment *Ss*SEB$_1$+*Pa*SEB$_{21}$ in the presence of the fungal pathogen which was significantly higher than all other treatments. The lowest root length was observed in the treatment with fungal pathogen only. Likewise, the plant height on bacterial inoculations showed an increase of up to 14.6% over uninoculated control and up to 76.6% over the treatment with *S. sclerotiorum* (Table 3). The highest plant height shown by the bacterial consortium *Ss*SEB$_1$+*Pa*SEB$_{21}$ was significantly higher than all other treatments. Fresh weight also showed significant variation within the treatments with the highest fresh weight shown by consortium *Ss*SEB$_1$+*Pa*SEB$_{21}$in the presence of the fungal pathogen (Table 3). Similarly, the dry weight also showed the same trend

with the highest dry weight shown by the consortium *Ss*SEB$_1$+*Pa*SRB$_{21}$ (Table 3).

The chlorophyll content of the plants treated with bacterial strains also showed a significant increase over the uninoculated control. The bacterial treatments also differed significantly from one another in increasing the chlorophyll content. The chlorophyll content varied from 0.183 to 0.37 mg/g with the highest content observed in the combined application of two strains *Ss*SEB$_1$+*Pa*SEB$_{21}$. The lowest chlorophyll content was observed in the treatment with only *S. sclerotiorum*. The total phenolic content of the plants also exhibited a significant increase with the bacterial inoculations over the control (Table 3). The TPC varied from 0.9 to1.49%, with the highest increase shown by the treatment *Ss*SEB$_1$+*Pa*SEB$_{21}$+*S. sclerotiorum*.

The disease control observed was up to 88.9% in bacterial inoculated plants (Table 3). However, no disease symptoms were observed in the uninoculated control and 100% disease incidences were observed in the treatment inoculated with only *Sclerotinia sclerotiorum* as evident by wilting, yellowing and breakage of stem and leaves of all plants.

Additionally, the nutrient content of sunflower plants (shoots) was also found to increase significantly with the bacterial inoculations over the uninoculated control with the highest N, P and K content shown by the treatment *Ss*SEB$_1$+*Pa*SRB$_{21}$in the presence of the fungal pathogen (Table 4). An increase of up to 56.7% in N content, 62.1% in P content and 65.8% in K content was observed with the *Ss*SEB$_1$+*Pa*SRB$_{21}$in the presence of the fungal pathogen over the control treatment *Sclerotinia sclerotiorum*. Likewise, the bacterial treatments also improved the physiochemical properties of the soil with a significant effect on the organic content of the soil (Table 4). The lowest organic carbon was shown by the treatment with the fungal pathogen *Sclerotinia sclerotiorum*. Similarly, a significant increase was observed in the phosphorous and potassium content of soil with the highest content observed in the combined treatment *Ss*SEB$_1$+*Pa*SRB$_{21}$in the presence of fungal pathogen *Sclerotinia sclerotiorum*.

Discussion

Fungal phytopathogens cause huge losses in almost every economically important crop. *Sclerotinia sclerotiorum* referred to as white mold is a broad host range fungal pathogen causing head rot, stem rot, watery soft rot and drop blight in several crops (Motlagh *et al*. 2023; Bornman *et al*. 2024). Endophytes produce a plethora of bioactive metabolites

which are helpful in the eradication of phytopathogens and thus, enhance growth and defense in plants. The present study was carried out to anticipate endophytic bacteria against *S. sclerotiorum* and to evaluate plant growth promoting attributes of these potential antagonistic bacterial endophytes.

In the present study, 12.5% of isolates (5 isolates out of 40) were found to inhibit the growth of *Sclerotinia sclerotiorum.* The results of the present study are in accordance with a previous study in which 18.6% of total isolated endophytic bacteria from medicinal plants, showed antagonism towards *Sclerotinia sclerotiorum* (Ansary *et al*. 2018). Likewise, 12.2% of endophytes obtained from sunflower and 12% obtained from soybean exhibited antagonistic potential against *Sclerotinia sclerotiorum* (Goes *et al*. 2012; de Almeida Lopes *et al*. 2018). *Pseudomonas* sp. showed growth inhibition of *Sclerotinia sclerotiorum* of up to 62-83% in dual plate assay (Deshwal 2012) and bacterial endophytes from *Stevia rebaudiana* leaves exhibited growth inhibition of *R. solani* ranging from 61 to 86% in liquid medium (Vyas and Singh 2024). However, information available on the antagonistic activity of bacterial endophytes of sunflower against *Sclerotinia sclerotiorum* is scarce.

Antagonistic bacterial endophytes are reported to inhibit fungal pathogen growth by damaging their hyphae and causing deleterious effects on their morphology (Vinayarani and Prakash 2018). In the present study, effects of antagonistic bacterial endophytes on fungal morphology studied by SEM analysis showed thinning, rupturing and intense breakage of fungal hyphae compared to healthy and condensed fungal hyphae in the control as also observed by earlier workers (Huang *et al*. 2019; Vyas and Singh 2024).

The bacterial strains were identified as *Sphingobacterium solani* SEB_1 and *Pseudomonas* sp. SEB_{21} by 16S rRNA gene sequence analysis. *Pseudomonas* spp. are well studied plant growth-promoting bacteria (Gulati *et al*. 2008; Bansal *et al*. 2025; Vyas *et al.* 2025). Different species of *Pseudomonas* including *P. corrugata*, *P. asplenii*, *P. mandelii*, and *P. protegens* have been reported to have inhibitory effects on growth of *S. sclerotiorum* in lettuce (Albert *et al.* 2024). Likewise, *Sphingobacterium solani* has recently been reported as a novel species to be isolated from the stem of potato plants growing in Hebei Province, China (Niu *et al*. 2018). However, *S. solani* has not been reported for any activity till now. In the present study, *S. solani* was reported as a strong biocontrol agent against *S. sclerotiorum* and a plant growth-promoting endophyte of sunflower for the first time. However, no reports are available

on the biocontrol potential of endophytic *Sphingobacterium solani* and *Pseudomonas* sp. isolated from sunflower against *Sclerotinia sclerotiorum*.

Volatile organic compounds produced by bacterial endophytes help in eradication and biofumigation of fungal pathogens. Furthermore, in the present study both *Sphingobacterium solani* SEB_1 and *Pseudomonas* sp. SEB_{21} were observed to produce 23 and 10 VOCs, respectively, with antagonistic properties (Fig. 4). The results of the present study are corroborated by earlier studies where Tetradecane, Hexadecane, 1-Nonadecane and 1-Octadecanol extracted from quinoa stem were reported for antifungal, antimicrobial, antioxidant and anti-inflammatory activities (Khan and Javaid 2019). Hexadecane extracted from essential oils have shown antimicrobial activity against *Aspergillus niger*, *Candida albican*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* (Das *et al*. 2016). Likewise, Tetradecane exhibited antifungal activity against pathogenic fungi such as *Aspergillus flavus* and *Rhizoctonia solani* (Mends *et al*. 2012; Karpagasundari and Kulothungan 2014). Similarly, 1-Nonadecene, 1-Octadecanol and 9-Hexacosene isolated from actinomycetes were found to inhibit growth of *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Botrytis cinerea* (Balachandar *et al*. 2018; Servi *et al*. 2019). VOCs may cause ROS accumulation, have inhibitory effects on biosynthesis of pyruvate and acetyl CoA, reduce intracellular ATP content and integrity of cell membranes of pathogens, thus showing antifungal properties against various phytopathogens (Zhang *et al*. 2021). In a recent study, volatile organic compounds produced by *Bacillus velezensis* TA-1 exhibit antagonistic effects against *Meloidogyne incognita* (Ji *et al*. 2024).

In the present study, isopropyl myristate, 3-hexadecene, 1-Nonadecane, 3-tetradecane, 1-octadecene, 2-4, and dihydroxyphenyland n-octadecanol are various VOCs produced by *Pseudomonas* sp. SEB_{21} and *Sphingobacterium solani* SEB_1. They have been reported to promote plant growth and play an important role in Induced Systemic Resistance (Kai *et al*. 2016). Similarly, volatile organic compound, tetradecane produced by *Bacillus velezensis* exhibited antifungal properties against *Verticillium dahliae* in tomato (Dhouib *et al*. 2019). Likewise, 2-4, dihydroxyphenyl showed antifungal properties against *Candida* sp. (Matysiak and Malinski 2007).

Furthermore, volatile organic compound production, *Sphingobacterium solani* SEB_1 and *Pseudomonas* sp. SEB_{21} also were observed to be positive for other attributes such as HCN, siderophore, ammonia production and hydrolytic enzyme activity, which may

be responsible for their strong antagonistic activity against *S. sclerotiorum* improved growth, chlorophyll content, total phenol content, and nutrient content as well as reduced disease incidences of *S. sclerotiorum* in sunflower. The results of increased N, P and K values of shoots over uninoculated control are in accordance with a previous study, in which a significant increase in nutrient content of leaves and seeds of sunflower was observed on inoculation with arbuscular mycorrhiza *Glomus hoi* (Gholamhoseini *et al*. 2013). The disease control (88.9%) observed in the present study was higher than a previous report where 6 out of 117 endophytic bacteria from rapeseed showed 64.7% growth inhibition of *S. sclerotiorum* in a pot trial (Sun *et al*. 2012).

Conclusions

The present study highlighted strong antagonistic activity of bacterial strains *Sphingobacterium solani* SEB_1 and *Pseudomonas* sp. SEB_{21} against *Sclerotinia sclerotiorum,* causal agent of stem rot in sunflower. Both bacterial strains showed production of VOCs along with the production of HCN, ammonia, siderophores and hydrolytic enzymes indicating their potential to be used as biocontrol agents. The synergistic interaction between both cultures, strengthen their biocontrol activity. Thus, a consortium of these strains exhibited plant growth promotion and disease suppression under greenhouse conditions. The formulation containing the consortium of these strains may be developed and tested in sunflower to control stem rot disease caused by *Sclerotinia sclerotiorum* in field conditions.

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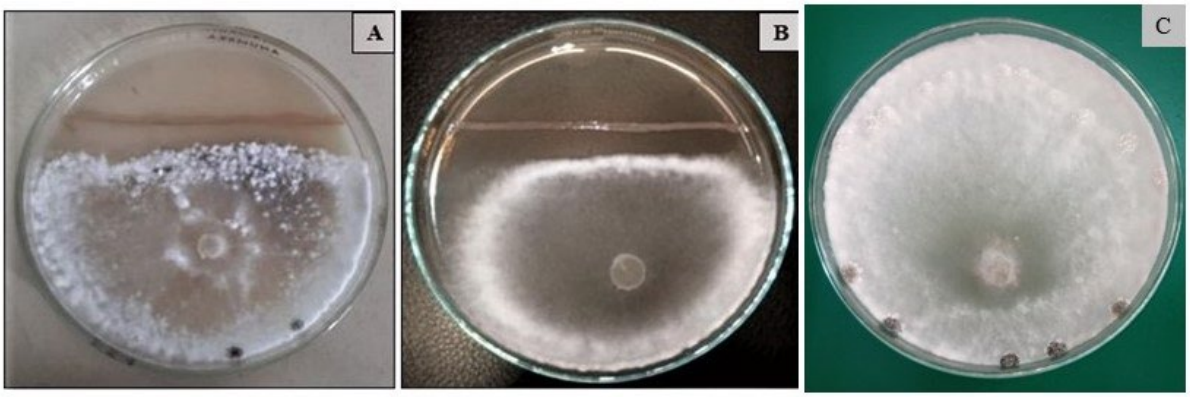

Fig. 1. Antagonistic activity shown by bacterial endophytes isolated from sunflower against *Sclerotinia sclerotiorum.* (A) SEB_1, (B) SEB_{21} and (C) Control.

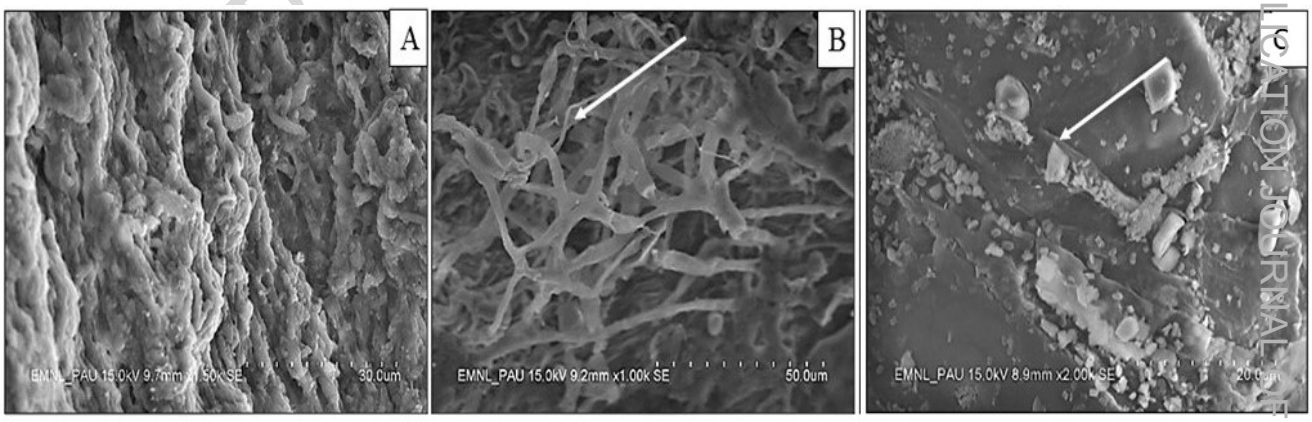

Fig. 2. Scanning Electron Micrograph of *Sclerotinia sclerotiorum* inoculated with bacterial endophyte (B) SEB_1, (C) SEB_{21} showing thinning and breakage of fungal hyphae in comparison to control (A).

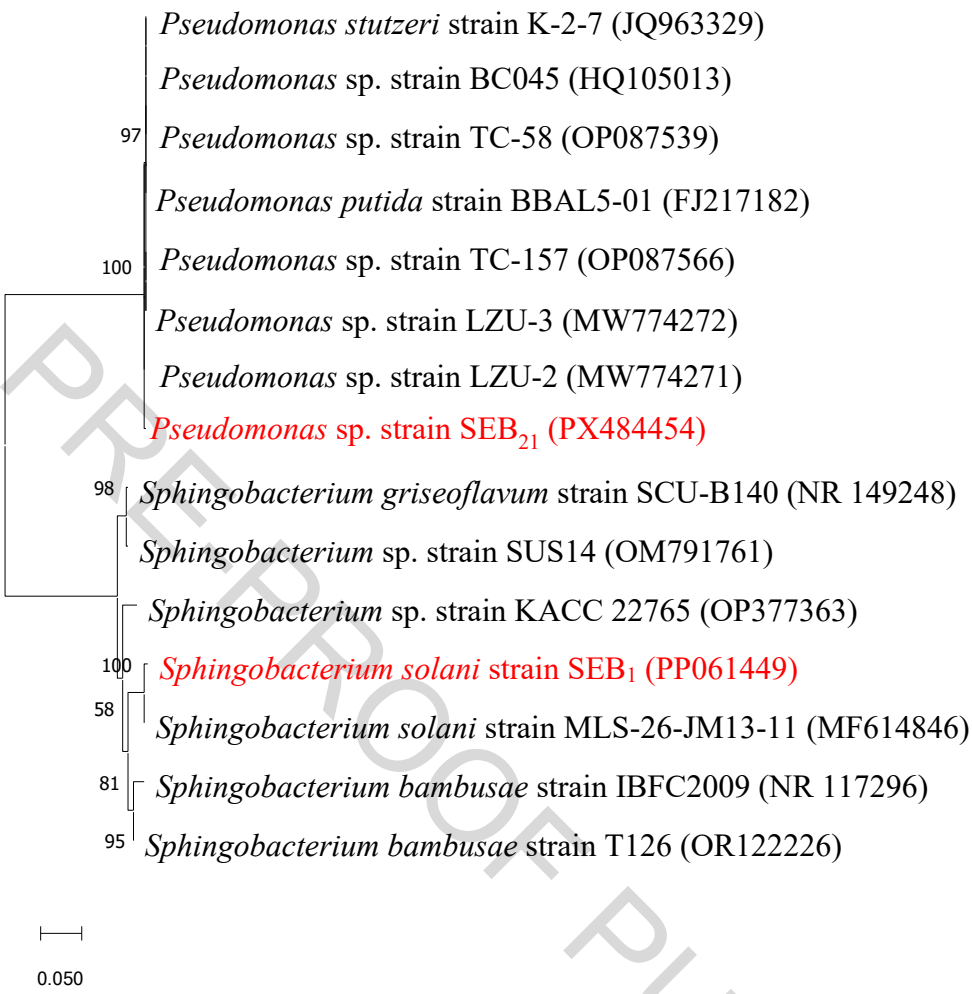

Fig. 3. Evolutionary relationships of antagonistic plant growth-promoting bacterial endophytes SEB_1 and SEB_{21} and their nearest neighbors based on the 16S rRNA gene sequencing using the Neighbor-Joining method elucidated using Mega version 11. The evolutionary distances were computed using the Kimura 2-parameter method.

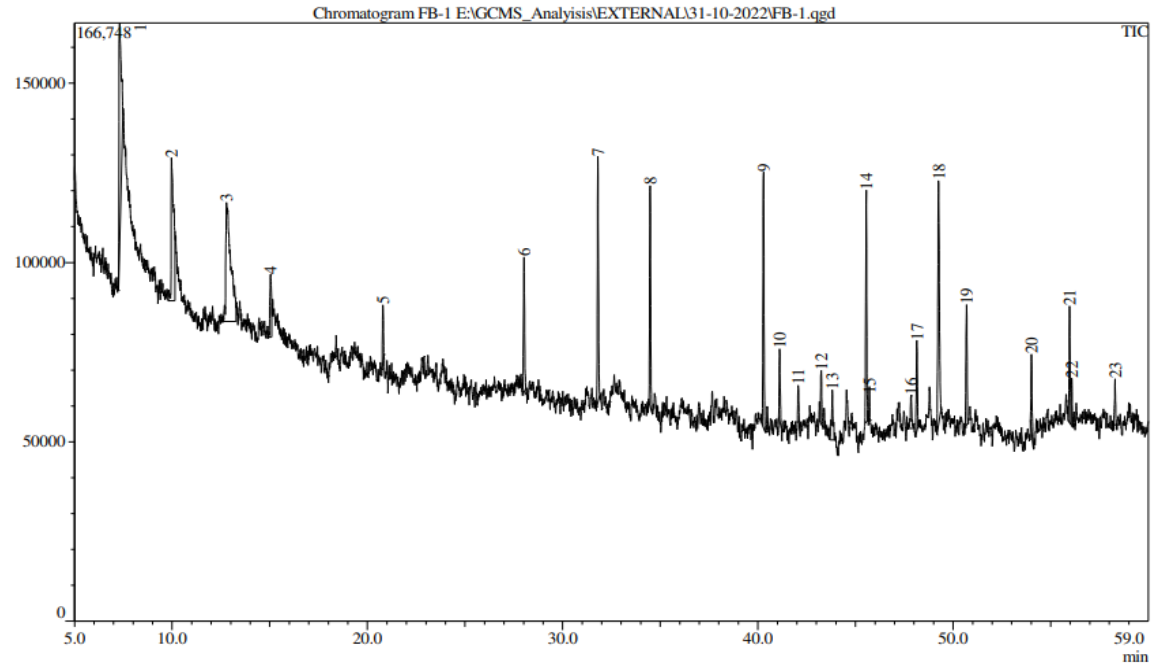

Fig. 4(a). Chromatogram representing peaks of volatile organic compounds produced by endophyte *Sphingobacterium solani* SEB_1. 1=Methylsufinylmethane, 2=Dimethyl-sulfone, 3=Phenol, 4=2-Hydroxybenzaldehyde, 5= Nonylcyclopropane, 6=3-Hexadecene, 7=2,4-ditertbutylphenol, 8-9=1-docoesne, 10=Isopropyl myristate, 11=1,2-Benzene-dicarboxylic acid, 12=7,9-Di-tert-butyl-1-oxosiro-4,5-deca-6,9-diene, 13=Hexadecanoic acid, 14= 1-Octadecene, 15= 2,6,10,15-Tetramethylheptadecane, 16=2,4-dihydroxyphenyl, 17=Tetratriacontane, 18=2,4-dimethylphenylimino-4-nitrophenyl,19=1-Nonadecene, 20= Octacosane, 21= n-octadecanol, 22=7-methylhexadecane, 23=Tritetracontane.

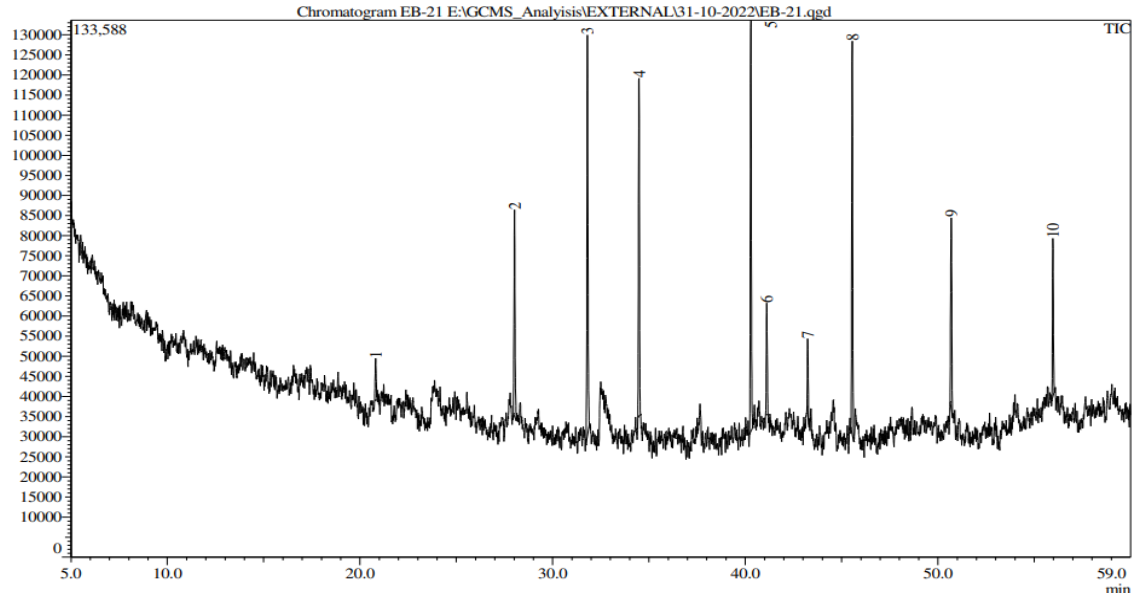

Fig. 4 (b). Chromatogram representing peaks of volatile organic compound produced by endophyte *Pseudomonas* sp. SEB_{21}.1=3-Tertradecane, 2=3-Hexadecene 3= 3,5-bis(1,1-dimethylethyl)-phenol, 4=3-Hexadecene, 5&8=1-octadecene, 6= Isopropyl myristate, 7=7,9-Ditert-butyl-1-oxospiro (4,5) deca-6,9-diene-2,8-dione, 9=1-tricosene, 10=n-octadecanol

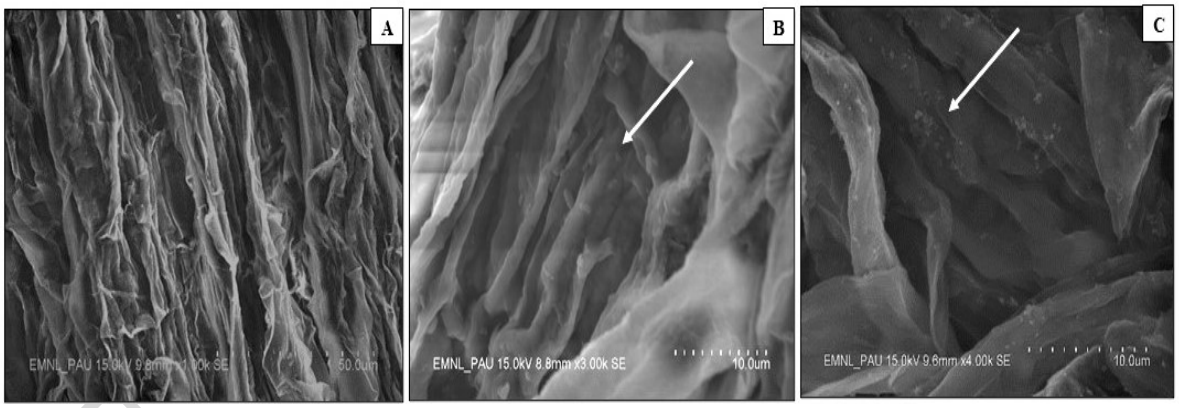

Fig. 5. Scanning Electron Micrograph of sunflower plant tissue. A. Control sample without any bacterial colonization, B. Colonization of plant sample by bacterial endophyte *Sphingobacterium solani* SEB_1 and C. *Pseudomonas* sp. SEB_{21}. Arrows indicate colonization by the bacterial endophytes.

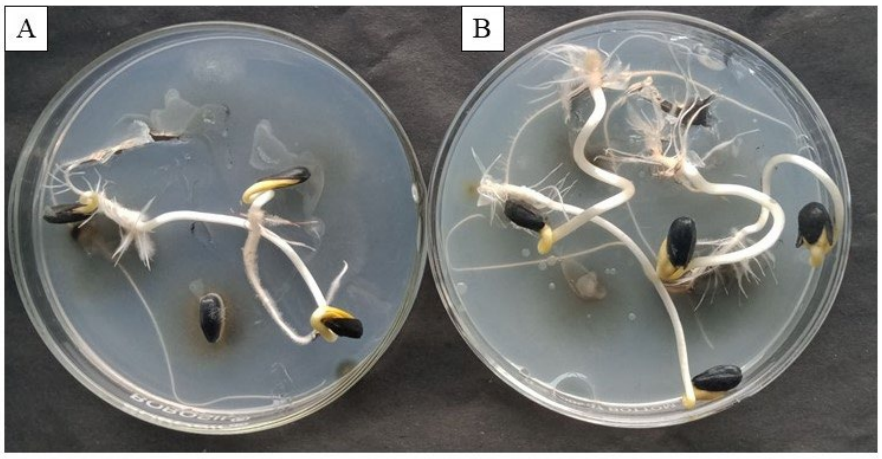

Fig. 6. Seed germination and root elongation assay. (A) Uninoculated control (B) Seed treated with bacterial endophyte SEB1

Fig. 7. Effect of antagonistic bacterial endophytes on the growth of sunflower. (A) Plants inoculated with endophytes without pathogen and (B) fungal pathogen inoculation on plants.

Table 1. Antagonism shown by bacterial endophytes isolated from sunflower against *Sclerotinia sclerotiorum* in liquid media

Treatment	Fungal biomass (DW, g)	Inhibition (%)
Control (*S. sclerotiorum)*	2.307[a]	0
SEB_1+ *S. sclerotiorum*	0.490[b]	78.7
SEB_{21}+ *S. sclerotiorum*	0.382[c]	82.0
SEB_1+SEB_{21}+ *S. sclerotiorum*	0.278[d]	88.1
CD at 5%	0.146	

Values represent mean of three replicates. DW: dry weight, CD: critical difference.Values with same letter do not differ from one another according to Duncan's Multiple Range Test at $p \leq 0.05$.

Table 2. Effects of antagonistic bacterial endophytes on germination and root elongation of sunflower on 1% water agar plates after 5 days of incubation at 28ºC

Treatment	Radical length	Germination (%)	Vigor index
Control	5.0±0.110	50.0	250.8[d]
SsSEB_1	6.1±0.160	75.0	457.5[b]
*P*spSEB_{21}	5.5±0.020	75.0	405.0[c]
SsSEB_1+*P*spSEB_{21}	6.8±0.330	75.0	510.0[a]
CD at 5%			44.06

Values represent the mean of 12 replicates (4 seeds per plate). Values with same letter in each column do not differ from one another according to Duncan's Multiple Range Test. *Ss Sphingobacterium solani* and *P*sp *Pseudomonas* sp.

Table 3. Effects of bacterial endophytes on growth parameters, total phenolic content, chlorophyll content and disease control of sunflower grown under greenhouse conditions

Treatment	Plant Height (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	TPC (%)	Chlorophyll (mg/g)	Disease incidence (%)	Disease control (%)
Uninoculated control (without fungal pathogen)	58.1±0.75^{b}	12.4±0.94^{b}	11.7±0.19^{b}	0.61±0.56^{b}	1.17±0.051^{bc}	0.250±0.025^{c}	0	-
Control with *S. sclerotiorum*	37.7±1.50^{c}	9.3±0.40^{c}	8.5±0.45^{c}	0.43±0.02^{c}	0.93±0.091^{c}	0.183±0.015^{d}	100	0
*Ss*SEB$_1$ +*S. sclerotiorum*	59.5±1.20^{b}	13.0±0.50^{b}	12.3±1.09^{b}	0.71±0.02^{a}	1.35±0.041^{ab}	0.310±0.020^{b}	22.22	77.9
*P*spSEB$_{21}$+*S. sclerotiorum*	59.3±4.60^{b}	12.6±0.65^{b}	12.1±0.99^{b}	0.65±0.01^{a}	1.25±0.076^{b}	0.34±0.077^{b}	22.22	77.9
*Ss*SEB$_1$+*P*spSEB$_{21}$+*S. sclerotiorum*	66.6± 1.22^{a}	14.9±0.84^{a}	13.8±1.17^{a}	0.77±0.03^{a}	1.49±0.325^{a}	0.37± 0.030^{a}	11.11	88.9
CD at 5%	4.26	1.27	1.58	0.06	0.28	0.34		

Values represent the mean of three replicates (9 plants). Values with same letter in each column do not differ from one another according to Duncan's Multiple Range Test. Ss *Sphingobacterium solani* and *P*sp *Pseudomonas* sp., TPC = Total Phenolic Content

Table 4. Effects of antagonistic bacterial endophytes on soil physico-chemical properties and nutrient content of sunflower grown in greenhouse

Treatment	Soil					Plant Nutrient Content (%)		
	pH	EC (dc/m)	OC content (Kg/ha)	Phosphorus (Kg/ha)	Potassium (Kg/ha)	Nitrogen	Phosphorus	Potassium
Uninoculated control (without fungal pathogen)	7.47± 0.032[a]	0.183±0.005[a]	0.343±0.002[b]	25.6± 0.10[a]	86.3±0.458[d]	1.05±0.032[c]	0.35±0.001[d]	5.6±0.015[c]
Control with *S. sclerotiorum*	7.31±0.026[a]	0.189±0.001[a]	0.284±0.004[c]	20.1±0.85[b]	77.5±0.450[e]	0.90±0.026[d]	0.29±0.006[e]	4.1±0.015[d]
*Ss*SEB$_1$ +*S. sclerotiorum*	7.50±0.015[a]	0.220±0.001[a]	0.379±0.003[a]	26.4±2.96[a]	111.0±0.152[b]	1.33±0.107[ab]	0.45±0.002[b]	6.1±0.058[b]
*P*spSRB$_{21}$+*S. sclerotiorum*	7.37±0.041[a]	0.218±0.001[a]	0.351±0.003[b]	26.0±3.91[a]	99.9±0.251[c]	1.18±0.045[b]	0.37±0.002[c]	5.8±0.052[c]
*Ss*SEB$_1$+*P*spSRB$_{21}$+*S. sclerotiorum*	7.40±0.031[a]	0.225±0.002[a]	0.397±0.003[a]	29.4±2.19[a]	126.9±0.251[a]	1.41±0.015[a]	0.47±0.010[a]	6.8±0.049[a]
CD at 5%	NS	NS	0.047	4.42	4.18	0.11	0.009	0.07

Values represent the mean of 3 replicates (9 plants). Values with same letter in each column do not differ from one another according to Duncan's Multiple Range Test. *Ss Sphingobacterium solani* and *P*sp *Pseudomonas* sp. EC = Electrical Conductivity, OC = Organic Carbon.