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Research Article

Rhizobial, passenger nodule endophytes and phyllosphere bacteria in combination with acyl homoserine lactones enhances the growth and yield of groundnut

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ABSTRACT

Quorum sensing (QS) mechanisms play an essential role in mediating several signals and plant-bacteria interactions, promoting plant growth. This study demonstrated production of multiple Homoserine lactone (HSL) molecules like C6 HSL, C7 HSL, C8 HSL, 3-Hydroxy-C8-HSL and 3-oxo-C14 HSL in rhizobial and passenger endophytes and phyllospheric bacteria which regulated production of plant growth promoting traits *viz.*, indole acetic acid and exo-polysaccharide production, biofilm formation, and motility. Quorum quenching (QQ) molecules like salicylic acid, gallic acid, and disalicylic acid impaired these traits, but exogenous addition of QS molecules (C7HSL and 3-oxo-C14 HSL) restored these inhibitory effects of QQ compounds. The pot culture experiment revealed that the treatment involving *Methylobacterium populi* TMV7–4 or *Enterobacter cloacae* S23 with salicylic acid, C7HSL and 3-oxo-C14 HSL significantly enhanced plant growth including root length, nodulation, pod formation, soil available nutrients and plant nutrients uptake. In future field validation is required for the use of QS molecules in improving groundnut production.

1. Introduction

Groundnut, a major oilseed crop, containing 38.83 - 56.48 % oil and 21.80 - 31.65 % protein [1] cultivated in semiarid regions of the world and are regarded as the fourth most valuable oilseed crop in the world [2]. According to a recent analysis, a number of biotic and abiotic variables, including an unfavourable environment, nutritional imbalance, bacterial and fungal-induced plant diseases, hinder groundnut production [3]. To increase the productivity, several conventional and biotechnological tools are being practiced which are hideous and very costly to adopt. Therefore, the use of microorganisms is being practiced for the recent years and are known to promote plant growth by production of several plant growth promoting traits and by promoting resistance to several stresses. On this context, the endophytic microorganisms of the groundnut plants that live inside the plant tissues helps the plants to overcome a variety of biotic and abiotic stresses [4]. The endophytes were categorized as nodule forming rhizobial and co-habituating passenger endophytes (RE and PE). The RE produces siderophores, indole acetic acid (IAA), ammonia, and ACC deaminase and efficiently solubilised phosphate [5–7] Whereas the PE like *Klebsiella* sp., *Pseudomonas putida, Klebsiella oxytoca, Enterobacter cloacae, Pantoea dispersa, Paenibacillus illionoisensis* and *Pseudomonas proteolytica*, from groundnut increase the plant growth, chlorophyll content, nodule count, shoot NPK content and soil physico-chemical properties [6–9]. While several phyllosphere inhabiting C1 compounds (methanol, methyl amine, and formaldehyde) utilizing pink pigmented facultative

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methylotrophs (PPFM) are involved in various plant growth promoting activities in several drought prone areas [10] by synthesis of phytohormones like IAA, ACC deaminase, siderophore production and also play major role in multiple stress related nutrient cycles [11].

In the recent years, few studies proved that the growth promotion by bacteria is mainly due to the production of quorum sensing (QS) compounds like N-acyl homoserine lactone (AHL), which are essential for the bacterial communication and to the interaction between host plant and bacteria [12–14]. Numerous QS-regulated genes, such as those involved in exopolysaccharide (EPS) [15], virulence, biofilm formation, chemotaxis, and many others, are activated or deactivated by the perception of QS molecules in bacteria [16,17] . They regulate the secretion of antibiotic compounds, siderophore, enzymes, virulence factors of phyto-pathogens, and plant-microbe communications [18]. One of the most significant classes of QS molecule is Acyl Homoserine Lactone (AHL) [19] which includes *N*-(hexanoyl)-l-homoserine lactone (C6-HSL), *N*-(3-hydroxyoctanoyl)-l-homoserine lactone (OHC8-HSL) and *N*-(3-oxododecanoyl)-l-homoserine lactone (OC12-HSL) which are majorly produced by *α*-, *β*- and *γ-Proteobacteriota* [17] whereas *Tenacibaculum maritimum* produces short-chain AHL (C4-HSL) molecule [20,21]. These QS signals are disrupted by some compounds namely, quorum quenching (QQ) molecules, encompassing very diverse phenomena and mechanisms [17,22]. The nature of QQ molecules varies based on the compounds involve in quenching of QS molecules including enzymes such as AHL-lactonase, AHL acylase and paraoxonase [23] which degrades the quorum sensing molecules and affects the pathogenic infections; cytochrome oxidases [22,24]. In contrast, the metallo-β-lactamase (LrsL) QQ enzyme, which possesses exceptional catalytic properties and a unique hydrophobic substrate binding pocket that can hold a variety of acyl-homoserine lactones (AHLs) with exceptionally high affinity, inhibited the formation of *Pseudomonas aeruginosa* biofilm without influencing bacterial growth and chemical compounds like 3-hydroxypalmitic acid methyl ester (3-OH-PAME) [25], salicylic acid, disalicylic acid, and gallic acid, as well as their mode of action, which includes QS-signal cleavage and competitive inhibition [26].

It is evident that QS plays a great role in the field of agriculture and human health [27]. Plants respond differently depending on the tissue to which they are exposed to plant growth promoting rhizobacteria (PGPRs) and AHLs throughout the colonisation process [28]. Application of pure AHLs changes root structure and growth, such as lateral root development, root hair production, primary root elongation [29] and enhanced nodulation [30]. The AHLs also confers the stress tolerance capacities [31], induction of systemic resistance by secretion of jasmonic acid and ethylene [32], and enhances the resistance toward bacterial and fungal pathogens [33]. The application of AHL compounds in tomato exhibited improved plant growth and resistance towards salinity [34]. Although the application of QS and QQ compounds are studied in the recent years, their application for the economical production of agricultural crops is yet be revealed. However, to date QS system in rhizobial and passenger endophytes has not fully explored. The current work was therefore designed with the following objectives: (i) to investigate the AHL molecules released by the bacteria associated with groundnut; and (ii) to show how QS mediates the expression of characteristics in these bacteria that promote plant growth. (iii) to elucidate the external application of QS molecules and bacteria improve the plant growth, yield, and uptake of nutrients by groundnut as well as the nutrients available in the soil.

2. Materials and methods

2.1. Selection of bacterial isolates and growth conditions

Four rhizobial endophytes *Rhizobium pusense* S6R2, *Rhizobium* sp. S19, *Rhizobium mayense* S11R1 and *Rhizobium phaseoli* S18; five passenger endophytes *Enterobacter cloacae* S23, *Burkholderia territori* KBB5, *Pantoea dispersa* YBB19B, *Burkholderia seminalis* TSB1 and *Paenibacillus illionoisensis* YBB20 [8] were selected based on our previous studies in groundnut [6–8]. Three phyllosphere pink pigmented facultative methylotrophic (PPFM) bacteria *Methylobactertium populi* TMV7–4, *Methylobactertium extorquens* VRI2–2 and *Methylobactertium extorquens* VRI2–7 were selected based on their groundnut plant growth promotion capacity as documented earlier by Krishnamoorthy et al. [35]. These bacterial isolates were obtained from the glycerol stocks stored at −80 °C from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. RE and PE, were cultivated in yeast extract mannitol, and nutrient broth at 28±2 °C for 24 h and PPFM were cultivated in glycerol peptone broth supplemented with 1 % methanol, at 28±2 °C for 48 h.

2.2. Extraction of AHL molecules from the bacterial isolates

RE, PE, and PPFM isolates were grown in their respective liquid medium supplemented with 50 mM 3-[N-morpholino] propane sulfonic acid (MOPS) to prevent the degradation of AHL [36] and incubated at 28 ±2 °C, 120 rpm for 24 h (RE and PE) and 48 h (PPFM). After centrifuging the bacterial cultures for 10 min at 12,000 rpm, 200 mL of the supernatant was extracted three times using an equivalent volume of ethyl acetate that had been acidified with 0.2 mL of glacial acetic acid [37]. The solvent fractions were separated and dried in a fume hood at room temperature and re-suspended in 1 mL of acetonitrile, and stored at −20 °C for further experiments. The acetonitrile extract was subjected to HPLC analysis for the detection of AHL molecules secreted by the bacteria.

2.3. Detection and quantification of AHL compounds

The presence of N-acyl homoserine lactone was assessed using a C18 column and a UV detector, the Shimadzu Nexera X2 HPLC system (Shimadzu, Japan). Acetonitrile and water (20–100 %) were used as the mobile phase. The effective flow rate and the temperature of the column were maintained at 1 mL min^{-1} and 40 °C, respectively. The experiment was conducted using a 20 μL injection volume and at 238 nm wavelength. Synthetic N-acyl homoserine lactones such as N-hexanoyl HSL (C6-HSL), N-heptanoyl HSL (C7-HSL), N-octanoyl HSL (C8-HSL), N-decanoyl HSL (C10-HSL), 3-Hydroxy-C8-HSL (3-OH-C8-HSL) and 3-oxo-C14 HSL (3-O-C14-HSL) were used as standards (Sigma Aldrich, USA) [38].

2.4. Determination of QS mediated plant growth promoting traits of bacterial isolates in the presence of QQ molecules

The QS mediated expression of plant growth promoting traits was determined by understanding the tolerance level of RE, PE and PPFM to the quorum quenching molecules like salicylic acid, gallic acid (5, 10 and 50 mM), and disalicylic acid (0.5 and 1.0 mM). The bacterial isolates were grown under different concentration of quorum quenching molecules. One percent inoculum of the selected isolates (1×10^8 cfu/mL) were inoculated in their respective liquid medium and incubated at 28 ±2 °C, 120 rpm for 24 h (RE and PE) and 48 h (PPFM). The growth was quantified by measuring their optical density at 660 nm by using a spectrophotometer (UV-1280, Shimadzu, Japan).

2.5. Indole acetic acid production by the bacterial isolates

The bacterial isolates were grown in 5 mL of their respective liquid medium containing 5 mM, 10 mM, and 50 mM concentrations of salicylic acid, gallic acid, and 0.5 mM and 1.0 mM of disalicylic acid amended with 0.1 % tryptophan for 48 h at 28±2 °C while being shaken (120 rpm) in order to determine the impact of the QQ molecules on the production of IAA. Then the cultures were centrifuged for 10 min at 12,000 rpm and the supernatant was used to estimate IAA. 500 μL of

supernatant was taken in a micro centrifuge tube to which 2 mL of Salkowski reagent (150 mL of concentrated H_2SO_4, 250 mL of distilled H_2O, 7.5 mL of 0.5 M $FeCl_3{\cdot}6H_2O$) was added, and the resulting combination was placed in dark condition for 30 min during the incubation pink to red colour development was observed. The colour intensity was measured spectrophotometrically (UV-1280, Shimadzu, Japan) at 530 nm [39] and standard graph was generated using pure IAA and expressed as μg/mL.

2.6. EPS production by the bacterial isolates

The EPS from the bacterial isolates was extracted using the ethanol precipitation technique since EPS synthesis was regulated by QS system. The bacterial isolates were cultured in their respective broth supplemented with QQ molecules and incubated at 28±2 °C for 24 h (RE and PE) and 48 h (PPFM). The cultures were centrifuged at 6000 rpm for 10 min. The supernatant was carefully transferred into new tubes, and an equal volume of 96 % cold ethanol (v/v) was added and allowed to stand for overnight precipitation. Then they were centrifuged for 20 min at 6000 rpm. The resulting pellets were dissolved in distilled water [40]. To the 1 mL of extracted EPS, 1 mL of 5 % phenol solution, and 5 mL of pure sulphuric acid, were added and the mixture was allowed to stand at room temperature for 30 min. The total EPS produced was estimated spectrophotometrically at 492 nm by measuring the brown colour intensity developed and the total EPS content was then calculated by plotting a standard graph with d-glucose ranged from 0 to 100 g/mL [41].

2.7. Biofilm formation by the bacterial isolates

The bacterial isolates ability to form biofilms in the presence of QQ molecules was assessed in order to determine how well they colonised host plants. Briefly, 10 μL (1×10^8 cfu/mL) of bacterial isolates were grown in a 96-well microtiter plate with 150 μL of their respective liquid medium supplemented with different concentrations of QQ molecules and kept for two-days incubation at 28±2 °C. In this experiment, uninoculated broth was served as a control. Then, the microtiter plates were washed using sterile distilled water and air dried to which 150 μL of 1 % crystal violet solution was added and allowed to stand for 45 min. The plates were subsequently washed twice with distilled water. The purple ring formation on the wells indicated the development of biofilm. The wells were filled with 200 μL of 95 % ethanol, and the intensity of the purple colour was measured spectrophotometrically at 590 nm using a microplate reader (Molecular Devices SpectraMax, USA) [42].

2.8. Swimming and swarming motility under the effect of QQ molecules

The swimming and swarming motility of bacterial isolates were evaluated using yeast extract mannitol agar, nutrient agar, and glycerol peptone agar media. To assess swimming motility, 0.3 % (w/v) agar was incorporated into each medium, while 0.6 % (w/v) agar was used for swarming motility assays. Additionally, 0.5 % (v/v) methanol was exclusively supplemented in the glycerol peptone medium to support the growth of *Methylobacterium*. 10 μL (1×10^8 cfu/mL) of the overnight grown cultures were spotted in the centre on their respective agar plates supplemented with different concentration QQ. After 24 h of incubation at 28 ± 2 °C, the motility of the bacterial isolates were analysed, measuring the extent of bacterial spread on plates containing QQ molecules [43].

2.9. Restoration of plant growth promoting traits

Based on the tolerance level and growth promotion activities of the selected bacteria, only four bacterial isolates were selected for the further experiments. The restoration of plant growth promoting traits was demonstrated in the presence of QQ and exogenous addition of QS molecules. Briefly, *R. pusense* S6R2, *E. cloacae* S23, *B. territori* KBB5, and *M. populi* TMV7–4 were cultured in their respective medium and supplemented each with 5 mM salicylic acid and 5 μM C7HSL and 3-oxo-C14 HSL. Further IAA, EPS, biofilm formation, swimming and swarming motility were determined as described previously.

2.10. The effect of QS and QQ molecules on root exudates

The groundnut seeds cultivated variety VRI 10 was obtained from Regional Research Station, Vridhachalam, Tamil Nadu Agricultural University. The seeds were surface disinfected in 70 % ethanol for 3 min and 2 % sodium hypochlorite for 1 min and washed with sterile water for five times. Then the surface disinfected seeds were soaked in accordance with following treatments, T1- *R. pusense* S6R2; T2- *E. cloacae* S23; T3- *B. territori* KBB5; T4- *M. populi* TMV7–4; T5- Salicylic acid (SA) (Quorum quenching molecule); T6- *R. pusense* S6R2 + SA; T7- *E. cloacae* S23 + SA; T8- *B. territori* KBB5 + SA; T9- *M. populi* TMV7–4 + SA; T10- *R. pusense* S6R2 + SA + C7HSL+ 3-oxo-C14 HSL; T11- *E. cloacae* S23 + SA+ C7HSL+ 3-oxo-C14 HSL; T12- *B. territori* KBB5 + SA + C7HSL+ 3-oxo-C14 HSL; T13- *M. populi* TMV7–4+ SA + C7HSL+ 3-oxo-C14 HSL; T14- SA+ C7HSL; T15- SA+ 3-oxo-C14 HSL; T16- Quorum sensing molecules C7HSL+3-oxo-C14 HSL; T17- Control for 2 h and subsequently sown in a funnel flask system. The setup comprised a sterile soil filled upper funnel section, while the funnel stem was packed with glass beads and sealed with perforated aluminium foil. The funnel stem was inserted into a 500 mL conical flask to facilitate the collection of root exudates. The entire set up was maintained for 45 days, during which the root exudates were collected in the bottom of the flask. The collected exudates were separated addition of equal volume of chloroform and shaken vigorously in a separating funnel and the solvent fraction was dried at room temperature and the concentrated solvent fraction was dissolved in 2 mL of HPLC grade methanol and processed for GC–MS analysis (Clarus SQ 8C GC–MS from Perkin Elmer, USA) [44].

2.11. The effect of QQ and QS molecules on growth, yield, soil and plant nutrients

The groundnut seeds were surface disinfected as previously described and subjected to above mentioned treatments (T1–T17) for 2 h. Subsequently, six seeds were sown in a mud pot with dimensions of 28 cm (top width), 16 cm (bottom width), and 25 cm (height), containing 10 kg of a soil mixture composed of soil, vermicompost, and sand in a 3:1:1 ratio. The initial chemical properties of the experimental soil were as follows: 245.70 kg/ha nitrogen (N), 20.67 kg/ha phosphorus (P), 517.92 kg/ha potassium (K), 25 ppm sulphur (S), 540.2 ppm copper (Cu), 820 ppm magnesium (Mg), with a pH of 8.4 and an electrical conductivity (EC) of 0.06 dS/m. The experimental setup was maintained for 95 days, after which phenological and yield parameters, including root length, shoot length, number of nodules per plant, number of pods per plant, and plant biomass content, were assessed. Additionally, available soil nitrogen, phosphorus, and potassium and plant nutrient uptake of nitrogen, phosphorus, and potassium were estimated. Available soil nitrogen was estimated by alkaline permanganate method through collecting the soil distillate (20 g of soil, 0.32 % potassium permanganate, 2.5 % sodium hydroxide) in 2 % boric acid and titrated against 0.02 N sulphuric acid using the double indicator (0.07 g methyl red and 0.01 g bromocresol green in 100 mL of 95 % ethanol) and expressed in mg/kg [45]. Soil available phosphorus was determined by Olsen method, where the filtrate (5 g soil in 50 mL of Olsen's reagent [0.5 M sodium bicarbonate adjusted to pH 8.5] for 30 min) was collected and mixed with 5 mL of molybdate reagent and 1 mL of the diluted stannous chloride solution [46] The available soil phosphorus was estimated spectrometrically by measuring the intensity of the blue colour developed at 660 nm and expressed as mg/kg. Available soil potassium was estimated by extracting 5 g of soil with 25 mL of 1 N ammonium acetate (pH 7.0) through shaking for 30 min, followed by

filtration. The filtrate was then analysed using a flame photometer and expressed in mg/kg [47]. Plant samples were collected, oven-dried at 65 °C, and sieved through a 0.5 mm mesh. One gram of plant sample was digested overnight with a diacid mixture (H_2SO_4:$HClO_4$ = 5:2) for nitrogen, while a triacid mixture (HNO_3:H_2SO_4:$HClO_4$ = 9:2:1) was used for phosphorus and potassium. Both mixtures were heated on a sand bath until a clear solution was obtained. The digest was diluted with distilled water, transferred to a 100 mL volumetric flask, and reserved for analysis. For nitrogen estimation, 10 mL of the diacid extract was neutralized with 4 0 % NaOH, and the released ammonia was collected in 2 % boric acid, followed by titration with 0.02 N sulphuric acid using methyl red–bromocresol green as an indicator. 5 mL of the triacid extract was mixed with 5 mL of Barton's reagent (*A* = 25 g ammonium molybdate in 400 mL H_2O, *B* = 1.25 g ammonium metavanadate in 300 mL of boiling water and A and B solutions are mixed and made up to a litre), and the yellow colour intensity was measured colorimetrically at 420 nm for phosphorus estimation. To estimate the potassium, 5 mL of the triacid extract was neutralised with ammonium hydroxide, diluted, and analysed using a flame photometer against a standard calibration curve. The nitrogen, phosphorus, and potassium concentrations were determined and expressed as mg/g [48].

2.12. Statistical analysis

All the experiments were carried out in triplicate and the data were mentioned with mean and standard error. The experimental data were subjected to analysis of variance (ANOVA) and significant differences among the means were tested with Tukey's HSD test calculated at $p < 0.05$. Statistical analyses were performed using R Software version 4.3.1.25. All graphs were created using Origin Software and R Studio. Network enrichment analysis was carried out using cytoscape software (Metscape version 3.6.1 software).

3. Results

3.1. Quantification of quorum sensing molecules produced by bacterial isolates

The production of AHLs by the bacterial isolates were quantified based on the peaks with corresponding standard AHLs chemicals through HPLC. The result revealed the secretion of AHLs like C6-HSL, C7-HSL, C8-HSL, 3-Hydroxy-C8-HSL and 3-oxo-C14 HSL by the tested bacteria. *R. pusense* S6R2, *R. mayens* S11R1, and *R. phaseoli* S18 synthesized C6-HSL and the phyllosphere bacteria *M. extorquens* VRI2–7 (403.9 μg/mL) and *M. extorquens* VRI2–2 (222.38 μg/mL) recorded with the maximum C6-HSL synthesis and all the selected bacterial isolates secreted C7-HSL except *M. extorquens* VRI2–7. C8-HSL was recorded in *R. pusense* S6R2, *Rhizobium* sp. S19, *M. populi* TMV7–4 and all passenger endophytes except *P. illionoisensis* YBB20. However, 3-Hydroxy-C8-HSL was detected in *M. extorquens* VRI2–7, *M. extorquens* VRI2–2, *P. illionoisensis* YBB20, *R. mayens* S11R1, and *R. phaseoli* S18. All bacterial isolates were reported to synthesize 3-oxo-C14 HS, with the exception of *R. mayens* S11R1 and *M. extorquens* VRI2–2 (Table 1; Supplementary Table 1 &2 and Supplementary Figure 1).

3.2. Growth of the bacterial isolates under the effect of QQ molecules

In the presence of varying concentrations of QQ molecules (*i.e.*, gallic acid, disalicylic acid, and salicylic acid), the spectrophotometric analysis showed that the growth of the chosen bacterial isolates was progressively reduced from lower to higher concentrations (5 mM to 50 mM concentrations) of gallic acid, salicylic acid, and 0.5 mM and 1 mM of disalicylic acid. *E. cloacae* S23 exhibited the maximum growth at 5 mM (1.44), 10 mM (1.23) and 50 mM (1.04) concentration of gallic acid. Furthermore *M. extorquens* VRI2–2 exhibited maximum growth (1.16 and 1.13) at 0.5 and 1 mM concentration of disalicylic acid, respectively (Supplementary Table 3).

3.3. Effect of QQ molecules on plant growth promoting traits

To evaluate the effect of quorum quenching molecules on the plant growth-promoting traits of bacterial isolates, we assessed IAA production using Salkowski reagent, EPS synthesis by ethanol precipitation method, biofilm formation through microtiter plate assay, and bacterial motilities including swimming motility on 0.3 % agar and swarming motility on 0.6 % agar. All the aforementioned characteristics were expressed at higher concentrations when QQ molecules were absent. At the lowest concentration, however, the manifestation of these features was considerably diminished by the addition of QQ molecules to their growth media.

The highest amount of IAA was produced by *E. cloacae* S23 at 5 mM concentrations of gallic acid (119.58 μg/mL), salicylic acid (124.41 μg/mL), and disalicylic acid (0.5 mM; 123.74 μg/mL). Among the rhizobial endophytes, *R. pusense* S6R2 showed the highest IAA content at 5 mM salicylic acid (74.74 μg/mL), gallic acid (103.82 μg/mL), and 0.5 mM (96.33 μg/mL) of disalicylic acid, whereas *M. populi* TMV7–4 showed the maximum IAA production at 5 mM salicylic acid (92.61 μg/mL) and 5 mM gallic acid (91.48 μg/mL), and *M. extorquens* VRI2–2 recorded 97.12 μg/mL IAA under 0.5 mM disalicylic acid.

The highest amount of EPS was produced by *E. cloacae* S23 at 0.5 mM

Table 1
Quantification of N-acyl homoserine lactone (AHLs) of rhizobial, passenger endophytes and phyllosphere bacteria.

Bacteria	Accession Number	N-acyl homoserine lactone (μg/mL)				
		C6 HSL	C7 HSL	C8 HSL	3-Hydroxy-C8-HSL	3-oxo-C14 HSL
Rhizobial endophytes						
Rhizobium pusense S6R2	MG569856	13.65±0.13^{d}	1.86± 0.04 ^{g}	0.15±0.00^{b}	ND	0.06±0.00^{e}
Rhizobium sp. S19	MT415399	ND	1.33±0.00 ^{g}	0.05± 0.00^{e}	ND	0.06± 0.00^{e}
Rhizobium mayense S11R1	MN044788	2.79± 0.01^{e}	1.33±0.03 ^{g}	ND	0.26± 0.00^{c}	ND
R. phaseoli S18	MT830893	0.10± 0.00^{e}	3.08± 0.07^{f}	ND	0.13± 0.00^{c}	1.82± 0.00^{a}
Passenger endophytes						
Pantoea dispersa YBB19B	MN032436	9.63± 0.03^{de}	5.68± 0.06^{e}	0.07± 0.00^{c}	ND	0.08± 0.00^{d}
Paenibacillus illionoisensis YBB20	MN032437	0.21 ± 0.00^{e}	24.39± 0.44^{b}	ND	0.06± 0.00 ^{c}	0.14± 0.00 ^{b}
Enterobacter cloacae S23	MN062622	74.44± 0.58^{c}	25.68± 0.17^{a}	0.32± 0.00^{a}	ND	0.03 ± 0.00^{fg}
Burkholderia territori KBB5	MN032380	ND	10.78± 0.17 ^{d}	0.15± 0.00 ^{b}	ND	0.05± 0.00^{ef}
Burkholderia seminalis TSB1	MN032385	ND	2.89± 0.06^{f}	0.06± 0.00^{d}	ND	0.02± 0.00 ^{g}
Phyllosphere bacteria						
Methylobacterium populi TMV7–4	KY882050	ND	6.37± 0.05^{e}	0.15 ± 0.00^{b}	ND	0.05± 0.00^{ef}
Methylobacterium extorquens VRI2–2	KY882058	222.38±4.28^{b}	16.40± 0.32^{c}	ND	18.52± 0.00^{a}	ND
Methylobacterium extorquens VRI2–7	KY882061	403.91±5.68^{a}	ND	ND	15.94±0.00^{b}	0.12±0.00^{c}

ND- Not detected .Values in each column represent the mean of three replicates ± standard error (SE) and the same letter(s) within the treatment are not significantly different from each other as determined by Tukey's HSD test ($p < 0.05$).

(100.31 μg/mL) of disalicylic acid, 5 mM of salicylic acid (111.59 μg/mL), and gallic acid (115.09 μg/mL). Maximum EPS formation was observed in *R. pusense* S6R2 (77.41 μg/mL at 5 mM salicylic acid), *R. mayense* S11R1 (93.25 μg/mL and 97.20 μg/mL at 0.5 mM disalicylic acid and 5 mM gallic acid, respectively), *M. extorquens* VRI2–7 (97.91 μg/mL at 0.5 mM disalicylic acid), and *M. populi* TMV7–4 (81.58 μg/mL and 90.55 μg/mL at 5 mM salicylic acid and gallic acid, respectively) (Table 2).

Under controlled conditions, all studied bacterial isolates showed stronger biofilm formation, with the exception of *R. mayense* S11R1, *P. illionoisensis* YBB20, and *M. populi* TMV7–4, which showed moderate biofilm formation. *R. pusense* S6R2, *R. phaseoli* S18, *P. dispersa* YBB19B, and all of the phyllosphere bacteria exhibited weaker biofilm production at 5 mM salicylic acid, whereas *Rhizobium* sp. S19, *R. mayense* S11R1, and *P. illionoisensis* YBB20 did not form biofilm. *M. populi* TMV7–4, *E. cloacae* S23, and *Rhizobium* sp. S19all formed more robust biofilm at 5 mM gallic acid. *E. cloacae* S23 and *B. seminalis* TSB1 developed stronger and weaker biofilms, respectively, while all bacterial isolates showed moderate biofilm development at 0.5 mM disalicylic acid (Fig. 1).

In the presence of 5 mM salicylic acid, the swimming and swarming motilities were considerably decreased, and neither swim nor swarm zones were seen in *M. populi* TMV7–4. and *R. pusense* S6R2. Under 5 mM salicylic acid, *B. territori* KBB5 had the largest swimming zone (8.8 cm), followed by *E. cloacae* S23 (8.7 cm) (Fig. 2).

3.4. Restoration of plant growth promoting traits by the external application of QS molecules

The most prevalent QS molecules, according to the HPLC analysis of the bacterial isolates, were 3-oxo-C14 HSL (except in *R. mayense* S11R1 and *M. extorquens* VRI2–2) and C7HSL (except in *M. extorquens* VRI2–7) (Table1). Therefore, by introducing these commonly found QS molecules into the culture medium of the isolates that contained QQ molecules at varying concentrations, the ability of these molecules to restore plant growth promoting characteristics was assessed.

The results showed that *E. cloacae* S23 produced higher IAA (125.56 μg/mL and 116.14 μg/mL), EPS (113.18 μg/mL and 98.56 μg/mL), and biofilm at 5 mM and 10 mM salicylic acid, respectively. Additionally, at 10 mM salicylic acid concentration, in addition to 5 μM C7HSL and 3-oxo-C14 HSL, *R. pusense* S6R2, *B. territori* KBB5, and *M. populi* TMV7–4 restored the plant growth promoting characteristics. *B. territori* KBB5 and *M.* populi TMV7–4 both produced weaker biofilms when exposed to 10 mM salicylic acid, while *R. pusense* S6R2 showed no biofilm production when exposed to 5 μM C7HSL and 3-oxo-C14 HSL (Table 3). When 5 μM of AHLs, specifically C7HSL and 3-oxo-C14 HSL, were added, all tested bacteria's swimming and swarming motility restored, with the exception of *M. populi* TMV7–4 (Fig. 2).

3.5. Effect of QS and QQ molecules on root exudates

The funnel flask experiment was used to evaluate how QS, QQ molecules and bacteria affect the groundnut crop's root exudates. The result revealed that seeds treated with *M. populi* TMV7–4 +SA + C7HSL+3-oxo-C14 HSL contained benzene, hexanal, cyclohexane, cyclohexylmethanol, carnegine, phenazepam and octanal. Acetic acid, hexanal, cyclohexane, octodecanoic acid compounds were found in the roots exudates of the seeds treated with *E. cloacae* S23 +SA+ C7HSL+3-oxo-C14 HSL. These substances have demonstrated important properties such as antiviral, antimicrobial, antioxidant, and medical and industrial uses. The provided network analysis demonstrated the interdependence of the organic acids that bacteria produce and their involvement in quorum sensing. Acetic acid, propanoic acid, and hexanoic acid are examples of organic acids that function as precursors for QS molecules such acyl-homoserine lactones, which are important mediators in bacterial communication.

Additionally, they affect quorum-sensing pathways by modifying nutrient solubilisation, biofilm formation, and pH—all of which are essential for bacterial interactions and community behaviour. Organic

Table 2
Plant growth promoting traits of groundnut bacterial isolates under varied concentration of quorum quenching molecules.

Bacteria	IAA production (μg/mL)				EPS Production (μg/mL)			
	Control	Salicylic Acid (5 mM)	Di salicylic Acid (0.5 mM)	Gallic Acid (5 mM)	Control	Salicylic Acid (5 mM)	Di salicylic Acid (0.5 mM)	Gallic Acid (5 mM)
Rhizobial endophytes								
Rhizobium pusense S6R2	106.24 ±0.006^{c}	74.74±0.00^{d}	96.33 ±0.003^{ab}	103.82 ±0.00^{b}	92.07 ±0.002^{k}	77.41±0.002^{i}	80.73±0.01^{fg}	79.56 ±0.002^{i}
Rhizobium sp. S19	82.16±0.00^{i}	71.10±0.00 ^{g}	73.71±0.001 ^{g}	75.36 ±0.001^{h}	104.38 ±0.003^{e}	69.83±0.04^{kl}	92.82±0.04^{cd}	90.33 ±0.001^{cd}
Rhizobium mayense S11R1	98.65 ±0.001^{e}	71.10±0.00 ^{g}	74.66±0.001^{f}	84.62 ±0.002^{e}	108.25 ±0.008^{d}	69.83±0 .07^{kl}	93.25 ±0.002^{cd}	97.20 ±0.005^{b}
R. phaseoli S18	88.93±0.00^{g}	72.38±0.00^{f}	74.007 ±0.002^{f}	75.75 ±0.001^{h}	100.56 ±0.003^{f}	70.53±0.03^{j}	92.66 ±0.003^{cd}	80.24 ±0.001^{gh}
Passenger endophytes								
Pantoea dispersa YBB19B	102.16 ±0.005^{cd}	71.10±0.00 ^{g}	71.45 ±0.001^{h}	71.40 ±0.003^{i}	114.34 ±0.07^{c}	80.67±0.03 ^{g}	71.79±0.001^{i}	69.83±0.02^{j}
Paenibacillus illionoisensis YBB20	96.45 ±0.005^{ef}	71.10±0.00 ^{g}	80.26 ±0.002^{d}	76.67 ±0.003^{f}	118.41 ±0.02^{b}	90.77±0.01^{d}	76.78 ±0.001^{h}	69.83±0.02^{j}
Enterobacter cloacae S23	126.12 ±0.007^{a}	124.41±0.01^{a}	123.74 ±0.002^{a}	119.58 ±0.003^{a}	120.69 ±0.01^{a}	111.59±0.02^{a}	100.31 ±0.002^{a}	115.09 ±0.002^{a}
Burkholderia territori KBB5	106.44 ±0.005^{c}	73.12±0.00^{e}	75.46±0.002^{e}	96.68 ±0.001^{c}	95.71±0.03^{ij}	83.56±0.09^{e}	80.42±0.00f^{g}	87.49 ±0.004^{ef}
Burkholderia seminalis TSB1	119.39 ±0.005^{b}	86.49±0.002^{c}	75.31±0.001^{e}	80.682 ±0.001^{f}	97.71 ±0.03^{gh}	91.04±0.01^{c}	80.63 ±0.001^{fg}	87.59±0.02^{ef}
Phyllosphere bacteria								
Methylobacterium populi TMV7–4	83.99 ±0.002^{h}	92.61±0.001^{b}	74.01±0.001^{f}	91.48 ±0.002^{d}	95.09±0.05^{ij}	81.6 ± 0.003$^{f\ h}$	90.55 ±0.001^{e}	90.55±0.004 ^{cd}
Methylobacterium extorquens VRI2–2	103.40 ±0.001^{d}	72.98±0.00^{f}	97.12 ±0.003^{b}	79.39 ±0.002 ^{g}	107.19 ±0.06^{de}	95.45±0.03^{b}	97.25±0.00^{bc}	80.48 ±0.002^{gh}
Methylobacterium extorquens VRI2–7	92.79±0.002 ^{f}	91.84±0.00^{b}	86.36±0.003^{c}	77.66 ±0.001^{h}	97.30 ±0.002^{gh}	80.38±0.003^{f}	97.91 ±0.002^{bc}	87.21 ±0.001^{ef}

Values in each column represent the mean of three replicates ± standard error (SE) and the same letter (s) within the treatment are not significantly different from each other as determined by Tukey's HSD test ($p < 0.05$).

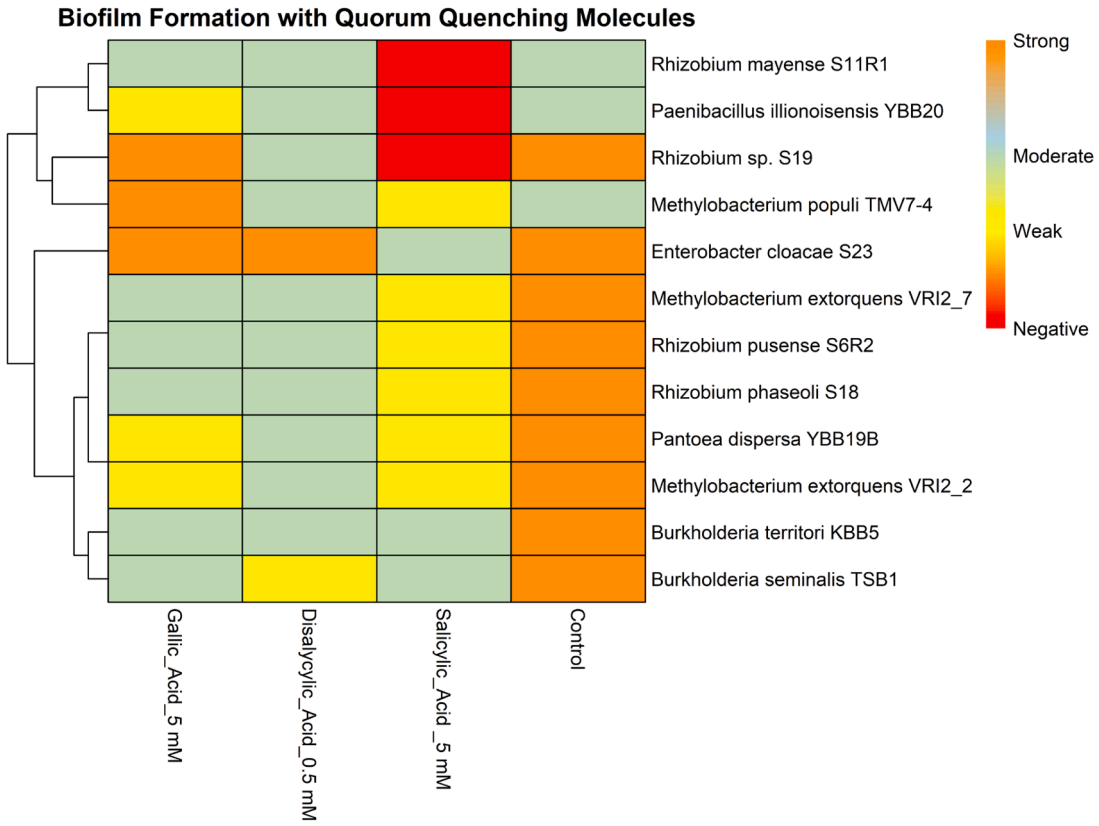

Fig. 1. Biofilm formation by groundnut associated bacteria in presence of various quorum quenching molecules. The heatmap illustrated the biofilm formation of different bacterial isolates under the influence of various quorum quenching molecules *viz*., salicylic acid (5 mM), disalicylic acid (0.5 mM), and gallic acid (5 mM). Different colours reflects the strength of biofilm formation.

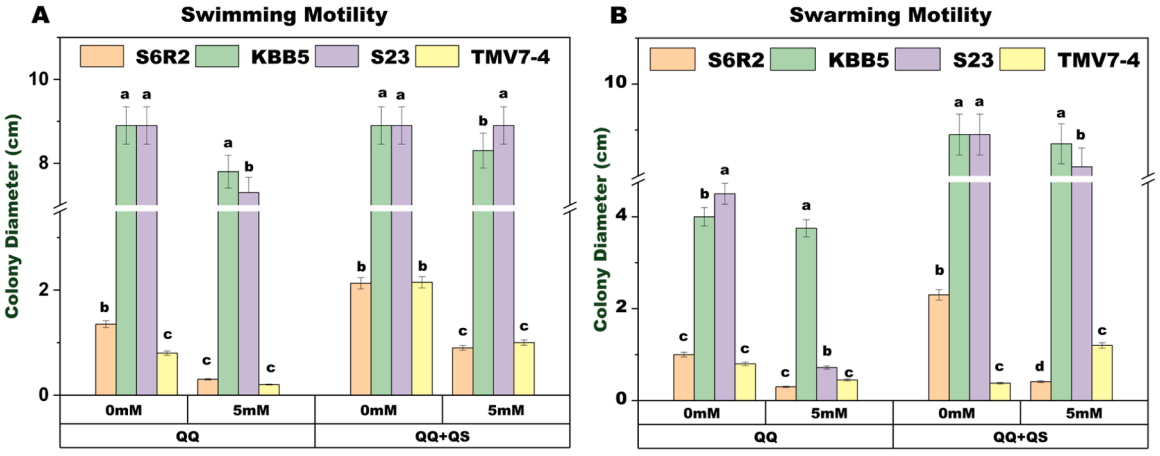

Fig. 2. Influence of QQ and QS molecules in swimming and swarming motility. The figure represents the impact of quorum quenching molecule (salicylic acid, 0 and 5 mM) and combination of salicylic acid with each 5 μM of C7-HSL and 3-oxo-C14 HSL on **(A)** swimming, **(B)** swarming motility of *R. pusense* S6R2, *B. territori* KBB5, *E. cloacae* S23, and *M. populi* TMV7–4 represented as colony diameter in cm. Error bars indicate the standard error (SE) of three replicates. The same letter (s) above bar are not significantly different from each other as determined by Tukey's HSD test ($p < 0.05$).

acids' intricate synergistic and antagonistic connections were highlighted by the diagram's dense network of positive (blue) and negative (red) correlations, highlighting their function in controlling bacterial activities like virulence, stress adaption, and resource competition (Fig. 3).

3.6. Role of QQ and QS molecules in the growth yield of groundnut and soil and plant nutrient contents

Groundnut was used as a test crop in pot culture conditions to evaluate the impact of QQ and QS molecules and bacteria on plant phenological and yield characteristics, including root length, shoot length, number of nodules, number of pods, and biomass content. The seeds treated with *M. populi* TMV7–4 +SA+C7HSL +3-oxo-C14 HSL exhibited longer roots (20.0 cm), the highest biomass (82.1 g plant^{-1}),

Table 3
Effect of quorum sensing molecules (C7HSL and 3-oxo-C14 HSL) on the plant growth promoting traits of selected bacteria under the influence of varied concentration of quorum quenching molecule (salicylic acid).

Bacteria	IAA production (µg/mL)		EPS Production (µg/mL)		Biofilm	
	5 mM	10 mM	5 mM	10 mM	5 mM	10 mM
Rhizobium pusense S6R2	74.67±0.002^{d}	71.11±0^{d}	78.31±0.003^{c}	69.83±0^{d}	Weak	Negative
Enterobacter cloacae S23	125.56±0.003^{a}	116.14±0.009^{a}	113.18±0.015^{a}	98.56±0.006^{a}	Strong	Weak
Burkholderia territori KBB5	80.28±0.004^{c}	77.38±0.003^{c}	85.95±0.004^{b}	78.43±0.002^{c}	Moderate	Weak
Methylobacterium populi TMV7–4	93.67±0.009^{b}	98.03±0.007^{b}	84.79±0.005^{b}	80.91±0.004^{b}	Weak	Weak

Values in each column represent the mean of three replicates ± standard error (SE) and the same letter (s) within the treatment are not significantly different from each other as determined by Tukey's HSD test ($p < 0.05$).
The optical density (OD) was measured at 590 nm using microplate reader. Strong >0.3, Moderate 0.2–0.29, Weak 0.1–0.19, Negative <0.10.

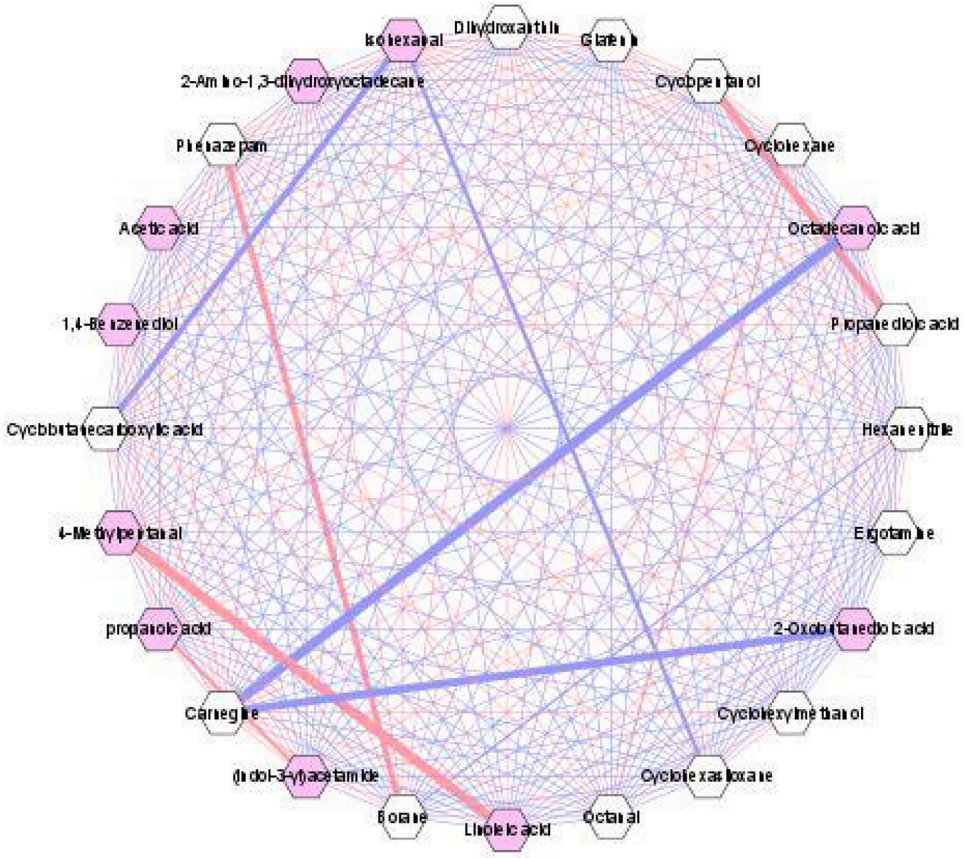

Fig. 3. Correlation analysis of metabolites obtained from the root exudates of groundnut associated bacteria. The resulting network visualization in Cytoscape represents compounds as nodes and significant correlations as edges, with edge thickness proportional to the correlation strength. The metabolites released from the different treatments T1-T17 were correlated and visualized using the Metscape app and the Cytoscape version 3.6.1 software. The blocks in white and purple represents the metabolites involved in various metabolisms as identified by Kyoto Encyclopedia of Genes and Genomes (KEGG) database/Human Metabolome Database.

and more nodules (167 nodules plant^{-1}). These were followed by quorum-sensing molecules C7HSL + 3-oxo-C14 HSL (15.3 cm), *E. cloacae* S23 +SA (68.9 g plant^{-1}), and salicylic acid alone (124 nodules plant^{-1}). However, the seeds treated with *E. cloacae* S23 (16.5 cm) and *R. pusense* S6R2 (16.0 cm) showed the longest shoots, which were on par with each other. More pods were formed by *E. cloacae* S23 +SA + C7HSL+3-oxo-C14 HSL (14 pods plant^{-1}), followed by *B. territori* KBB5 +SA + C7HSL+3-oxo-C14 HSL (13 pods plant^{-1}) (Fig. 4& 5).

The treatments that contained *R. pusense* S6R2 + SA (464 kg/ha N), *E. cloacae* S23 + SA + C7HSL + 3-oxo-C14 HSL (23.3 kg/ha P), and *M. populi* TMV7–4 (450 kg/ha K) increased levels of soil available nutrients. However, treatments using the quorum-sensing compounds C7HSL+3-oxo-C14 HSL (150.3 kg/ha N), salicylic acid (10 kg/ha P), and *M. populi* TMV7–4 + SA (120 kg/ha K) showed the lowest soil nitrogen content. In terms of plant nutrient uptake, the highest total NPK nutrients were noted in *E. cloacae* S23 + SA + C7HSL + 3-oxo-C14 HSL (59.3 mg/g N; 7.6 mg/g P; 37.9 mg/g K, respectively). The treatments with the lowest NPK contents were 10 µM quorum-sensing molecules C7HSL (41.9 mg/g N); control (5.5 mg/g P); and *B. territori* KBB5 +SA + C7HSL+3-oxo-C14 HSL (30.5 mg/g K) (Fig. 6).

4. Discussion

The intricate dynamics of quorum sensing and quorum quenching mechanisms, and their implications for promoting plant growth in groundnut were examined in this study. This study demonstrated the detection and characterisation of a diverse array of N-acyl homoserine lactones synthesized by rhizobial, passenger endophytes and

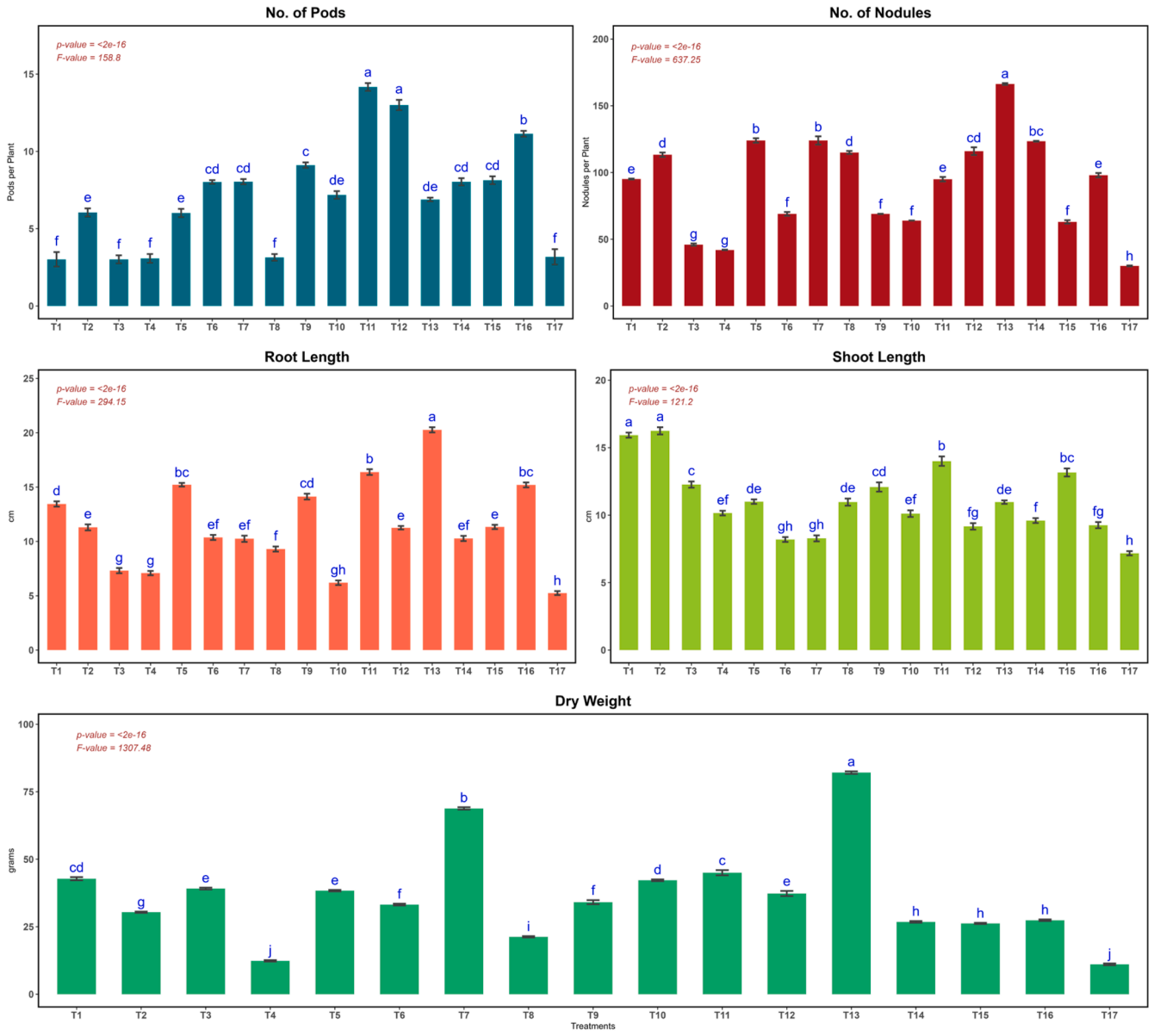

Fig. 4. Effect of bacterial isolates, QS and QQ molecules on growth and yield of groundnut. The figure illustrates the growth parameters of groundnut plants *viz*., the number of pods, nodules, root length, shoot length, and dry weight under pot culture conditions. **T1**: *R. pusense* S6R2 alone; **T2**: *E. cloacae* S23 alone; **T3**: *B. territori* KBB5 alone; **T4**: *M. populi* TMV7–4 alone; **T5**: Salicylic acid alone (quorum-quenching molecule); **T6**: *R. pusense* S6R2 + SA; **T7**: *E. cloacae* S23 + SA; **T8**: *B. territori* KBB5 + SA; **T9**: *M. populi* TMV7–4 + SA; **T10**: *R. pusense* S6R2 + SA + C7HSL + 3-oxo-C14 HSL; **T11**: *E. cloacae* S23 + SA + C7HSL + 3-oxo-C14 HSL; **T12**: *B. territori* KBB5 + SA + C7HSL + 3-oxo-C14 HSL; **T13**: *M. populi* TMV7–4 + SA + C7HSL + 3-oxo-C14 HSL; **T14**: Quorum-sensing molecule C7HSL (10 μM); **T15**: Quorum-sensing molecule 3-oxo-C14 HSL (10 μM); **T16**: Quorum-sensing molecule C7HSL + 3-oxo-C14 HSL; **T17**: Control. Values represent the mean of three replicates ± standard error (SE). The same letter (s) above bar are not significantly different from each other as determined by Tukey's HSD test ($p < 0.05$).

phyllosphere methylotrophic bacteria (PPFM). In this investigation, the selected isolates' AHL production was carried out using their corresponding growth media that had been amended with MOPS. MOPS solution was added to growth media to maximize extraction efficiency and shield AHLs from lactonolysis at alkaline pH [37,49,50], Through the comprehensive analysis, we aimed in unravelling the multifaceted roles of QS and QQ compounds in shaping the plant-microbe interactions which are critical for the groundnut growth and development. For instance, previous researches have elucidated the AHL synthesis capabilities of different rhizobial strains, such as *R. leguminosarum* bv. *viciae* produced AHLs like C6 AHL and 3OC8 AHL [51]. *Sinorhizobium fredii* HH103 has been reported to produce a range of AHLs including C8-HSL, 3-oxo-C8-HSL, C12-HSL, C14-HSL, and 3-oxo-C14-HSL [52] and *Sinorhizobium meliloti* synthesized multiple AHLs together with C6 AHL and 3OC14 AHL [53] which were similar to the production of AHLs like C6 HSL, C7 HSL, C8 HSL, 3-Hydroxy-C8-HSL and 3-oxo-C14 HSL produced by the selected RE, PE and PPFM isolates in this study. *E. cloacae* have been found to produce short-chain AHLs like C6-HSL and C8-HSL in addition to longer chains like C10-HSL, 3-oxo-C12-HSL, and C14-HSL, according to a study by İpek [50]. Furthermore, members of the *Burkholderia cepacia* complex (Bcc) have been found to predominantly produce C8-HSL and C6-HSL [54], including *Burkholderia* sp. A9 [55]. Early reports highlighted the AHL production patterns in *Methylobacterium* species, such as the detection of 3OHC14-HSL in *M. populi* P-1 M, and C8-HSL, and C6-HSL in *M. extorquens* [56] whereas *M. extorquens* isolate VRI2–2 produced C6 HSL, C7 HSL and 3-Hydroxy-C8-HSL in the present study. The main reason for the varying concentration is due to the secretion of short chain AHLs and long chain AHLs biosynthesis [57].

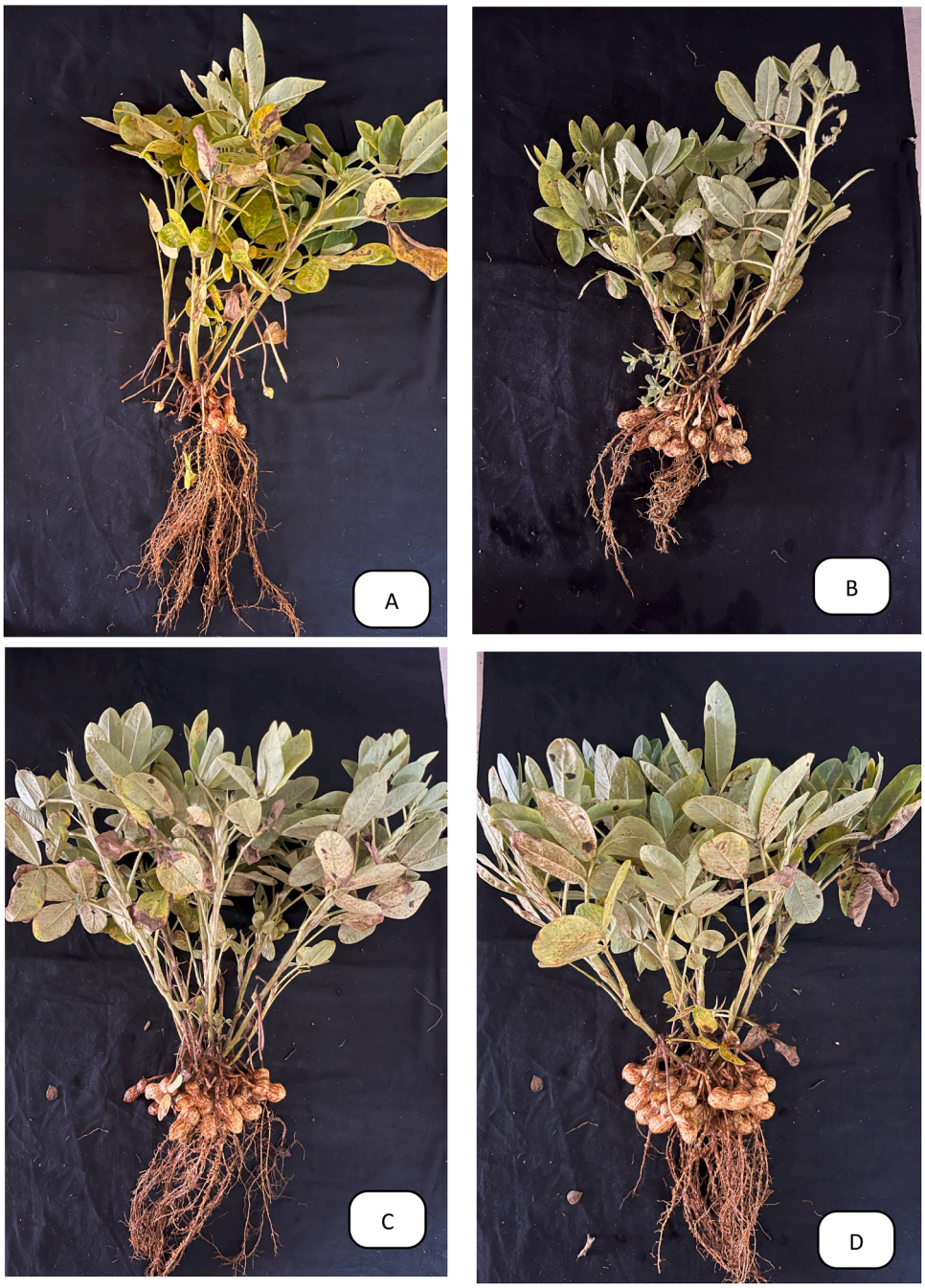

Fig. 5. Visual effect of QS molecules in the growth and yield of groundnut. A. Control (untreated) **B**. Treated with *E. cloacae* S23 **C**. Treated with *E. cloacae* S23 + SA + quorum sensing molecules (C7-HSL and 3-oxo-C14 HSL), **D**. Treated with *Enterobacter cloacae* S23 + salicylic acid (SA).

AHL compositions were specifically determined by the effects of environmental variables, such as pH, substrate concentration, and C/N ratio [58–60]. The weak signals detected for the N-acyl homoserine lactone molecules in the samples analysed could be due to various factors, such as chemical degradation, metabolic degradation, or enzymatic inactivation of these molecules [61]. In the present study, small peak was noted in the chromatogram for the few bacterial isolates and one way to correct this type of result is to increase the concentration of the extract to be analysed and modify the characteristics of the column [62]. In the present study, HPLC technique was employed to quantify the AHL production. As there were no significant impurities interference in the AHLs observed at the retention times of both the extracted samples and AHL standards, this method can be considered to be specific for AHL determination [50]. Through genetic engineering, AHL synthase genes such as luxI homologs can be overexpressed, and bacteria can be cultivated in environments with limited iron to boost their production of AHL [63].

In this study the QQ molecules such as salicylic acid, gallic acid and disalicylic were observed to reduce the activity of the bacterial isolates which are on par with the findings of Borges et al. [64] where the addition of gallic acid and ferulic acid reduced the activity of the *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Listeria monocytogenes*. Since quorum sensing controls the production of bacterial EPS, which, in turn, play an important role in biofilm formation. It provides structural support to biofilms, making them resistant to shear forces, facilitates the adsorption of various metal, organic, and inorganic compounds, as well as regulate mass transfer within the biofilm [65]. They are also responsible for cell adhesion, cell aggregation

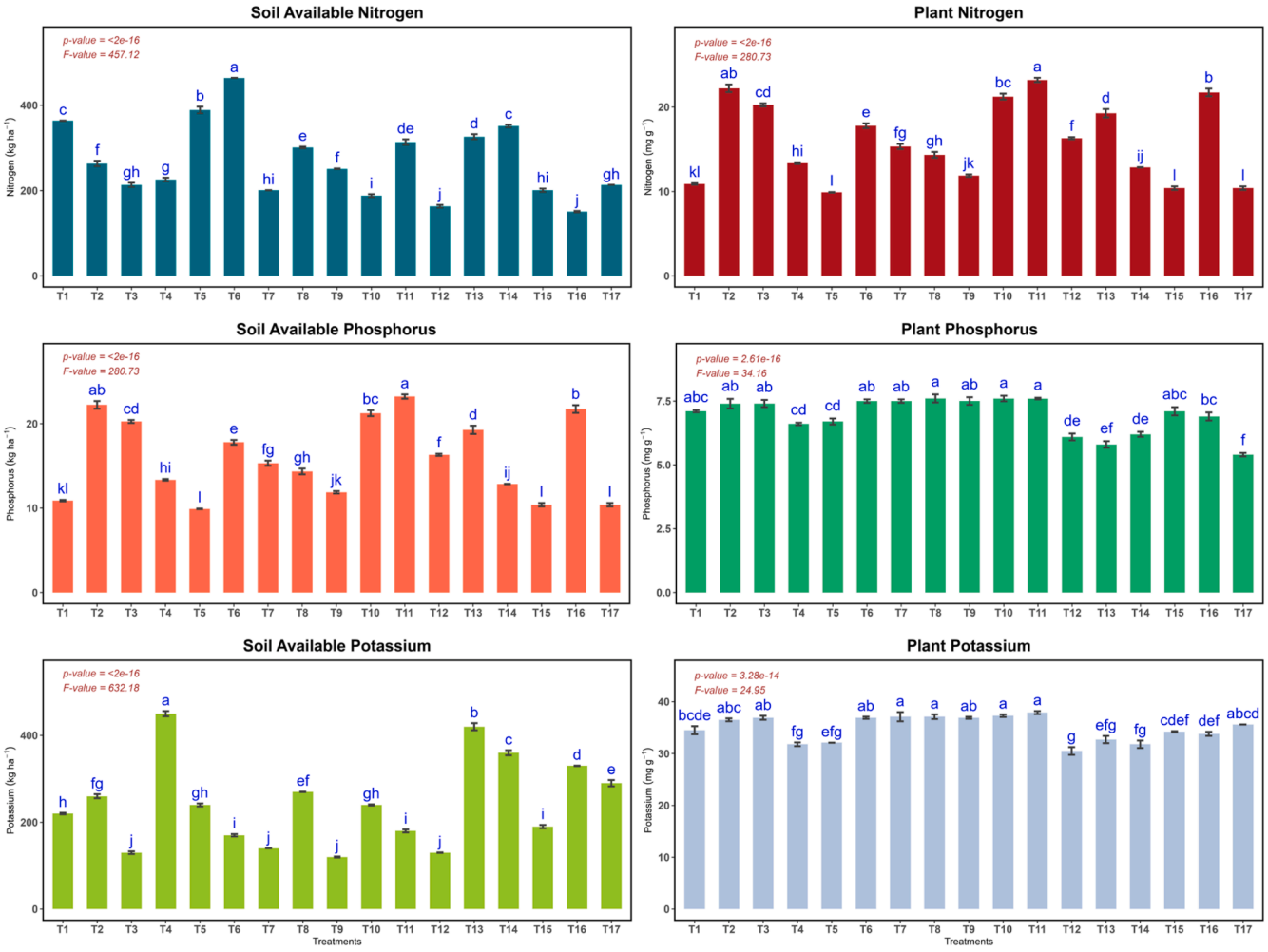

Fig. 6. Effect of bacterial isolates, QS and QQ molecules on soil and plant nutrients. The figure represents available nitrogen (N), phosphorus (P), and potassium (K) in soil and nutrient uptake in plants. **T1**: *R. pusense* S6R2 alone; **T2**: *E. cloacae* S23 alone; **T3**: *B. territori* KBB5 alone; **T4**: *M. populi* TMV7–4 alone; **T5**: Salicylic acid alone (quorum-quenching molecule); **T6**: *R. pusense* S6R2 + SA; **T7**: *E. cloacae* S23 + SA; **T8**: *B. territori* KBB5 + SA; **T9**: *M. populi* TMV7–4 + SA; **T10**: *R. pusense* S6R2 + SA + C7HSL + 3-oxo-C14 HSL; **T11**: *E. cloacae* S23 + SA + C7HSL + 3-oxo-C14 HSL; **T12**: *B. territori* KBB5 + SA + C7HSL + 3-oxo-C14 HSL; **T13**: *M. populi* TMV7–4 + SA + C7HSL + 3-oxo-C14 HSL; **T14**: Quorum-sensing molecule C7HSL (10 μM); **T15**: Quorum-sensing molecule 3-oxo-C14 HSL (10 μM); **T16**: Quorum-sensing molecule C7HSL + 3-oxo-C14 HSL; **T17**: Control. Values represent the mean of three replicates ± standard error (SE). The same letter (s) above bar are not significantly different from each other as determined by Tukey's HSD test ($p < 0.05$).

and water retention while forming a protective barrier and providing nutrient sources [66] and an important mediator in maintaining plant heath in abiotic stress [67]. It is one of the key traits responsible for colonisation of bacteria in plants and hence EPS production by the bacterial isolates were determined in this study. In order to identify the effect EPS production in the presence of QQ compounds Rehman et al. [68] conducted a study, which revealed that the QQ enzyme, LrsL suppressed the exopolysaccharides production required for biofilm formation. This effectively inhibited the biofilm formation of *Pseudomonas aeruginosa* without affecting the growth. The current study revealed the addition of QQ compounds reduced the activity of all the selected isolates and their production of EPS, biofilm and IAA. In contrast, the production of IAA in *R. pusense* S6R2 was most likely unaffected by QS probably it is regulated by host contact, enzymatic regulation, and substrate availability rather than QS alone [69]. Also, the biofilm production was impaired with the addition of salicylic acid, which strongly supressed the QS regulation in *R. pusense* S6R2 [70]. The results of this investigation are supported by a study that found *Xanthomonas oryzae* pv. *oryzae*'s EPS was completely suppressed at 200 ppm of salicylic acid [58].

The QS mechanism is known to regulate the production of biofilms in bacteria [71], while QQ compounds are responsible for inhibiting this process [58]. In this study there is a significant reduction in the biofilm formation at 0.3 and 0.4 mM of disalicylic acid, while salicylic acid significantly reduced biofilm development at a concentration of 0.6 mM and, to a higher extent, at 1.0 mM and 1.5 mM [72]. Similarly, inhibition of biofilm formation was reported in *Pectobacterium carotovorum* 29 and *Pseudomonas syringae* pv. *syringae* 13 [73] and in *Pseudomonas aeruginosa* [74]. In the current study, gallic acid's inhibitory effects on *P. dispersa* YBB19B and *R. phaseoli* S18 biofilm development were comparable to the 60.47 % inhibition of biofilm production in *E. coli* [75].

Quorum sensing is essential for controlling bacterial cell population density, directing motility toward plant roots, and coordinating the shift from motility-associated pathways to nodulation processes [76]. Investigation in *Xanthomonas oryzae* pv. *oryzae* revealed that the addition of salicylic acid to the growth medium resulted in a concentration-dependent reduction in swimming motility diameter, highlighting the potential of small molecules in disrupting QS-regulated behaviour [77]. The swimming and swarming motilities were documented in *P. aeruginosa* [78,79] where the cells swim in the semisolid medium by means of flagella, and forms the biofilm [80]. The QQ molecules *viz.*, gallic acid and ferulic acid at 1000 mg/mL were observed

to interfere the swimming and swarming motilities of *P. aeruginosa* [64]. In the present study, the motility of all the selected isolates were altered with the addition of salicylic acid. The motility of *R. pusense* S6R2 and *M. populi* TMV7–4 was completely retarded with the addition of salicylic acid. However, a higher salicylic acid concentration was found to decrease the motility of *B. territori* KBB5 and *E. cloacae* S23. The motile cell senses the stimuli from the environment and alters their functioning to improve its chances of migrating to a better location [81]. Therefore, the addition of QS compounds to the QQ affected environment reverts the activity of the QS stimuli by which the suppressed activity of motility was reverted. In this study the addition of 5 μM C7HSL and 3-oxo-C14 HSL increased the motility of *B. territori* KBB5 and *E. cloacae* S23.

In the current study, the application various quorum quenching and quorum sensing molecules together with beneficial endophytes produced several root exudates compounds like carbohydrates, amino acids, organic acids, and secondary metabolites, which can influence the microbial community surrounding the root system and promote beneficial plant-microbe interactions for plant growth [82]. *Enterobacter cloacae* S23 +SA+ C7HSL+3-oxo-C14 HSL-treated plant root exudate included acetic acid, a phytohormone also referred to as a plant growth regulator that also had antifungal activity against *Colletotrichum falcatum* [83].

In agreement with our findings, *Methylobacterium komagatae* enhances the root area in *Crambe abyssinica*. According to earlier research, the AHL molecule C8 HSL encourages root elongation in barley [29]. This finding is consistent with the current study, which found that groundnut roots lengthened considerably after being treated with the AHL molecules C7HSL and 3-oxo-C14 HSL. Similar results were obtained in the study conducted by Suprapta et al. [84] on rice seedling when applied with *Enterobacter cloacae* which enhanced the shoot length.

In an earlier study *M. oryzae* elevated the root and shoot dry weight in lentils [85] and 5 and 10 μM of C6-HSL and oxo-C10-HSL boosted the dry mass. However, we have experienced greater dry weight when groundnut seeds are treated with C7HSL and 3-oxo-C14 HSL along with *M. populi* TMV7–4 and salicylic acid. The nodulation potential of *Crotalaria juncea* was enhanced by inoculating it with *Macroptilum atropurpureum* and *Methylobacterium nodulans* [86]. The number of nodules in *Medicago truncatula* increased when 1 μM of 3-oxo-C14-homoserine lactone was added [30]. Additional research on *Arabidopsis thaliana* showed that 10 μM of C6-HSL treatment improved the IAA-to-cytokinin ratio, increased indole-3-acetic acid levels, and lengthened roots [87]. These results led to the selection of a total concentration of 10 μM for this investigation, which included 5 μM of C7-HSL and 5 μM of 3-oxo-C14-HSL.

The current study found that treatments containing QQ and QS molecules improved plant parameters such as root length and nodules in *Methylobacterium populi* TMV7–4 +SA + C7HSL+3-oxo-C14 HSL and pods in *Enterobacter cloacae* S23 +SA + C7HSL+3-oxo-C14 HSL compared to a control group that received only one of the components. *Enterobacter* species are known phosphate solubilisers, producing extracellular polymeric substances and siderophores [88]. In the current study the treatments involving *R. pusense* S6R2 and salicylic acid, resulted in the maximum soil available nitrogen due to its ability to fix atmospheric nitrogen within the nodules, making it readily accessible for plant utilisation while minimising the depletion of soil nutrients. Additionally, the plants treated with two quorum sensing molecules demonstrated higher nitrogen uptake compared to those with lower soil available nitrogen, indicating that these molecules enhance nitrogen absorption from the soil. This trend was also observed with potassium uptake and its availability in the soil when plants were treated with *E. cloacae* S23, salicylic acid, C7HSL, and 3-oxo-C14 HSL. The QS molecules such as C6-HSL, 3-oxo-C10-HSL, 3-OH-C5-HSL, and 3-oxo-C7-HSL were known to improve nutrient cycling process in a various crops like wheat, pea, soybean, and mung bean [89,90]. These QS molecules involve in the processes of root colonisation and nodulation that are essential for symbiotic nitrogen fixation [91]. These compounds enhanced root development and nitrate reductase activity in wheat [89] involved in nodule formation in peas and soybeans [90] that enrich ammonia uptake by plants by activating nif-genes especially under saline stress conditions [89]. *Cin*I gene present in *Rhizobium leguminosarum* were reported to be involved in the production of long-chain QS compounds that are crucial for nitrogen fixation in legumes [90]. Salicylic acid, being an elicitor molecule, enhanced the growth of groundnut while seeds were primed together with salicylic acid and bacterial isolates. This result was supported by the study conducted by Liang et al. [92], where the salicylic acid hydroxylase genes (AhS5H1 and AhS5H2) reduced the salicylic acid level in groundnut plants causing the plants susceptible to pathogens. Salicylic acid also plays a major role in regulating plant cells and their growth through various mechanisms [93].

5. Conclusion

This study reported the presence of AHL molecules like C6 HSL, C7 HSL, C8 HSL, 3-Hydroxy-C8-HSL, and 3-oxo-C14 HSL from the rhizobial, passenger and phyllosphere bacterial isolates of groundnut. The quorum-sensing (QS) mediated plant growth promotion was investigated through the application of QQ molecule like salicylic acid, gallic acid and disalicylic acid which impaired the QS signals and affected the plant growth promoting traits of these bacterial isolates. Whereas the addition QS molecules like C7HSL and 3-oxo-C14 HSL to the bacterial isolates under the influence of quorum quenching (QQ) molecules, had shown reversion in their impaired plant growth promoting traits. It was evident from the pot culture study that the application of QS molecules like C7HSL and 3-oxo-C14 HSL along with salicylic acid and bacterial isolate or salicylic acid with bacterial isolate have shown to enhance the growth and yield metrics. This work provides valuable insights into bacterial signalling and its potential application in agricultural biotechnology, particularly in developing bio-based strategies for crop production. Further, the molecular mechanisms revealing the interaction between QQ and QS signals in plant systems are yet to be investigated. The QS molecules knock-out mutant of these bacteria are also required to understand the exact effect of QS molecules in the growth promoting ability of bacteria. It is necessary to investigate the features of the QS molecules released by these bacterial isolates as well as their complex function in suppressing QQ signals in the plant system. In practical applications, the AHL mediated promoting impact can be achieved by selection of appropriate synthetic AHL and to develop biosensors that identify the AHL and QQ molecules in the soil. Furthermore, field-level research is necessary to comprehend how QS molecules support plant growth and to adjust the dosage of seed priming.

Author agreement

To certify that all authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the author's original work, hasn't received prior publication, and isn't under consideration for publication elsewhere.

Ethics approval and consent to participate

Not applicable.

Consent for publication

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CRediT authorship contribution statement

Sivakumar Madhan: Writing – review & editing, Writing – original draft, Visualization, Software, Formal analysis. **Yuvasri Errakutty Arunan:** Writing – review & editing, Software, Formal analysis. **Anandham Rangasamy:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Balachandar Dananjeyan:** Validation, Supervision, Data curation. **Johnson Iruthayasamy:** Supervision. **Manimaran Gajendiran:** Visualization. **Krishnamoorthy Ramasamy:** Supervision, Methodology. **Raghu Rajasekaran:** Supervision, Data curation. **Vincent Saminathan:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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Data availability

Data will be made available on request.

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