MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

The response of Vicia faba growth, pigmentation and secondary metabolites to plant growth-promoting bacteria

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Received:20/2/2025
Accepted: 10/3/2025

Abstract: The use of plant growth promoting bacteria in agricultural application for various purposes is of great significance. In this study, three bacterial strains *Pseudomonas MAP5*, *Bacillus lecheniformis* and *Bacillus aerius* nominated for their ability to grow on naphthalene as a sole carbon source in addition to their potentiality as plant growth promotors. The treatment of *Vicia faba* seeds with *Pseudomonas MAP5* resulted in 2.6 times higher shoot length (17.9 cm) compared to the control (13.3 cm, $p = 0.0001$). Treatments with *Bacillus lecheniformis* boosted chlorophyll (a) contents by 1.2 times (0.56 mg/g f. wt.) compared to the control (0.47 mg/g f. wt.) ($p = 0.0001$). Plant electrolyte leakage showed substantial reduction, specifically after *Bacillus aerius* treatment (16.71%) compared to control (28.17%, $p = 0.0001$). Additionally, the phenolic content increased notably in *Rhizobium*-treated plants, reaching (12.18 mg GAE/g d. wt.) above the control level (8.95 mg GAE/g d. wt., $p < 0.0001$), which indicates these bacterial strains potentially enhance plant growth and stress adaptation capability. The results represented in this study indicated the potentiality particularly of *Bacillus lecheniformis* as bean growth promotor and secondary metabolites modulator paving the way for their application for the bioremediation of polycyclic aromatic hydrocarbons.

keywords: Bacillus, bioinoculants, faba beans, pigmentation, Pseudomonas MAP5.

1.Introduction

Agriculture is the art and science of cultivating the land, producing food, fiber, and other essential commodities necessary for human sustenance and economic prosperity. However, despite its fundamental importance, agriculture faces a multitude of obstacles and problems that challenge its sustainability and global impact. Farmers face many obstacles including soil erosion, climate change, rising demand, biodiversity loss, consumer preferences, low productivity, global warming, and lack of financial resources [1, 2].

Using plant growth-promoting bacteria (PGPB) in agriculture is a promising strategy to enhance crop productivity and sustainability and considered one of the most important ways to overcome many agriculture challenges. PGPB is the more eco-friendly and long-term option for boosting plant growth and health [3]. PGPB are a class of beneficial bacteria that can stimulate plant development and growth through processes like nitrogen fixation, phosphorus solubilization, and hormone synthesis. Because of their potential for fostering sustainable agriculture and low environmental impact, PGPBs have attracted much attention as bioinoculants in recent years. They can improve plant growth and health through nutrient solubilization, hormone synthesis, and disease suppression.

PGPB can solubilize minerals like phosphorus and iron, making them accessible to plants and increasing nutrient absorption [4]. In addition, they can convert atmospheric nitrogen into a form that plants can use [5]. PGPB promotes plant growth by secreting chemicals that stimulate plant development, such as auxins, cytokinins, and gibberellins [6]. Their

organic acids can help root development by breaking down soil particles and releasing nutrients [7]. Antibiotics and other chemicals produced by PGPB can prevent the spread of plant diseases [8]. For instance, the antibiotic bacillomycin is produced by *Bacillus subtilis* and has been shown to defend plants against fungal infections [9]. Chemical fertilizers and insecticides can be used less frequently when farmers employ PGPB [10]. This has the added benefit of enhancing soil health and decreasing pollution levels. To further improve plant development and soil health, PGPB can be used with other management practices, such as crop rotation and conservation tillage [11].

PGPBs are an excellent resource for environmentally friendly farming because of their many benefits and qualities. Improve nutrient uptake, plant development, disease suppression, chemical input reduction, and compatibility with other management practices are all possible with their use. To give plants nutrients, chemical fertilizers are commonly utilized. Unfortunately, they can cause soil degradation, contamination, and harm to the soil's microbiota [12]. Because of PGPB, fewer chemical fertilizers are required for plant growth [7].

One of the agricultural challenges also is the polycyclic aromatic hydrocarbons (PAHs) which represent a group of organic compounds consisting of multiple fused aromatic rings. Some common examples of PAHs include naphthalene and anthracene. PAHs are often formed during the incomplete combustion of organic materials, such as wood, coal, and petroleum. PAHs can also be present in the environment, because of natural processes like forest fires and volcanic eruptions [13]. PAHs are of environmental and health concern because many of them are considered persistent organic pollutants (POPs) and are known to be potentially harmful to both human health and the environment. Some PAHs are classified as carcinogens, and prolonged exposure to them has been linked to various health problems, including cancer [14]. Efforts are made to regulate and reduce the emissions of PAHs in industrial and environmental settings and to limit its harmful effects on plants and humans. Among the most important one of these efforts is bioremediation using plant growth promoting bacteria, it considered as a crucial strategy in addressing PAH contamination [15].

PGPB enhances the degradation of PAHs in soil and water environments by various mechanisms, including the production of enzymes such as dioxygenases, that can metabolize PAHs into less toxic compounds [16]. It has been successfully applied in field studies to remediate PAH-contaminated sites, that demonstrates the effectiveness of using PGPB in real-world scenarios for PAH degradation. [17].

Environmental pollutants consisting mainly of polycyclic aromatic hydrocarbons (PAHs) pose a rising danger to agricultural productivity as these pollutants remain in soils and disrupt plant growth and metabolic processes. The challenge needs sustainable, eco-friendly solutions for promoting plant health and environmental contamination prevention. Plant growth-promoting bacteria (PGPB) demonstrate value through two key mechanisms: they promote nutrient uptake and growth enhancement and effectively degrade harmful organic pollutants, including PAHs.

The findings of this study serve as dual solutions for agricultural sustainability and formulation of environmental remediation. The research examines the effects *Pseudomonas MAP5*, *Bacillus lecheniformis*, and *Bacillus aerius* have on enhancing *Vicia faba* growth and photosynthetic capacity and biochemical functionality when exposed to pollution from PAHs. The wide acceptance of PGPB for nutrient solubilization, hormone synthesis, and stress tolerance exists alongside scarce research into their ability to enhance resistance against organic pollutants in plants. The research on the combined effects of *Rhizobium* with bacterial species to degrade PAHs while evaluating their consequences on plant physiological reactions remains poorly investigated in existing literature.

This study tests the assumption that PGPB strains that use naphthalene as their sole carbon source will substantially improve *Vicia faba* growth and physiological characteristics through enhanced nutrient acquisition, stress protection, and secondary metabolite regulation. The application of bacteria should also lead to reduced electrolyte leakage,

elevated chlorophyll content, and antioxidant defense mechanisms that support plant stress tolerance. The research investigates these factors to develop fundamental knowledge about how to employ PGPB to maximize plant productivity and foster environmental health.

This research moves insights into microbial-assisted phytoremediation and demonstrates how PGPB could be applied sustainably in agriculture. The research displays how single bacterial strains effectively double plant growth performance through both biological activity and PAH degradation, thus creating preparation possibilities between biotechnology applications for agricultural and environmental management.

Therefore, the aim of this study is to test the effect of (PGPB) plant growth promoting bacteria for their ability to degrade naphthalene on *Vicia faba growth* parameters, photosynthetic pigment percentage, membrane features and secondary metabolites content.

2. Materials and methods

Materials:

Proline and ninhydrin were obtained from Sigma (https://www.sigmaaldrich.com/). Other chemicals were of analytical grade and were purchased from several local companies.

2.1. Selection of the bacterial isolates

We started with 10 bacterial strains and assessed their ability to degrade naphthalene. The growth test was committed in a basal salt media (BSM) containing 1 g NH_4Cl, 0.33 g $Na_2HPO_4.2H_2O$, 0.38 g $NaH_2PO_4.H_2O$, 0.1 g $MgCl_2.7H_2O$, 0.1 g $CaCl_2.2H_2O$, 0.04 g KCl and 0.001g $FeSO_4.7H_2O$ in one-liter dist. water. The media was supplemented with 0.012 g naphthalene sachets fixed on the lid of the petri dish. The incubation takes place at 37°C till growth development. After screening all the tested strains, the following three bacterial ones showed their ability for naphthalene degradation:

Pseudomonas MAP5 **(P5)** isolated from (*Phaseolus vulgaris* nodules) with accession number (MG214654) [18].

Bacillus lecheniformis **(X1)** isolated from (*Calligonum*-associated lichen, *Xanthoria parietina*) with accession number (MW450665) [19].

Bacillus aerius **(X3)** isolated from (*Calligonum*-associated lichen, *Xanthoria parietina*) with accession number (MT035879) [19].

2.2. Pot experiments:

A pot experiment was designed to investigate the influence of the test strains and *Rhizobium* on broad bean plants' growth, pigmentation and metabolism. The experiments were run with a homogeneous lot of *Vicia faba seeds* (Sakha 1) along with *Rhizobium* sp., They were obtained from the Agricultural Research Center, Ministry of Agriculture, Egypt. The seeds came from the same batch and were picked for their apparent homogeneity in size. The plastic pots used in this study had a height of 20 cm and a top diameter of 25 cm, ensuring adequate space for root development and plant. The pots were contained 5 kg mixture of sand and clay (2:1). *V. faba* seeds were subjected first to surface sterilization using 0.02% sodium hypochlorite for 2 min. Afterword, seeds were washed by sterile distilled water.

The seeds were divided into separate groups, and each was subjected to the following bacterial treatments as shown in the next scheme as observed in Table (1)

Table (1). Experimental design

Treatment codes	Treatment description
T1	Control (without any bacteria)
T2	*Rhizobium*
T3	P5 + *Rhizobium*
T4	X1 + *Rhizobium*
T5	X3 + *Rhizobium*

(P5) for *Pseudomonas MAP5*, (X1) *for Bacillus lecheniformis and (*X3) for *Bacillus aerius.*

Experimental design and sampling

The three bacterial strains utilized in the previously described treatments were taken from fresh LB plates and cultured in LB liquid media at 37°C and 150 rpm and the concentration were adjusted to 10^8 CFU ml^{-1}. The necessary volume of cells was centrifuged at 6000 rpm for 20 min at 4°C to get rid of the culture media. After re-suspension in tap water the seeds were soaked for 45 mins. After bacterial inoculation, each subgroup was made up of 10 pot replicates. Ten seeds were then planted in each pot, buried about 1 cm below the soil's surface. During the experiment, the pots were maintained in the greenhouse of the

Faculty of Science, Mansoura University under typical day and night circumstances. The *Vicia faba* samples were collected at 21 days of age and subsequently stored at 4°C for further analysis.

2.2.2. Morphological, physiological and biochemical estimates of the selected samples

The collected samples were analyzed to determine the following growth parameters: water percentage of the shoot and root; length of the shoot and root; fresh and dry weight of the shoot and root; leaf area; number of nods; number of nodules; pigment (chlorophyll a, b, total chlorophylls, carotenoids, and total pigment). Additionally, membrane features; membrane injury index (MII), membrane stability index (MSI), electrolyte leakage and proline, total phenolic and flavonoid content were all assessed.

2.2.2.1. Determination of chlorophyll and Carotenoids contents

Following the method proposed by Hiscox and Israelstam [20], the chlorophyll carotenoids contents were estimated. At room temperature for 24 hours, 7 milliliters (mL) of dimethyl sulfoxide (DMSO) were used to extract 0.1 g of powdered leaves. After filtering the optical density was measured using Jenway spectrophotometer at 470, 644 662 nm (UK). Using the provided equations, the Chl.a, Chl. b, total Chl. and carotenoid contents of the sample plants were determined.

Chl. a $=12.7\times OD_{662}-2.69\times OD_{644}$ mg/L

Chl. b $= 22.9\times OD_{644}-2.69\times OD_{662}$ mg/L

Total Chl. $= 20.2\times OD_{644}+8.02\times OD_{662}$ mg/L

Carotenoids $= 5.02\times OD_{470}$ mg/L

2.2.2.3 Estimation of membrane features

Electrolyte leakage (EL)

The electrolyte leakage (EL) was estimated in fresh plant leaf tissues (one cm^2 pieces away from the midrib). The electrical conductivity (EC1) was measured after placing the tissue in a test tube containing 10 mL dist. water. The EC2 was measured after the tubes were shaken for 2 hours. After autoclaving, EC3 reading was taken after cooling down [21].

Electrolyte leakage (%) = (EC2 – EC1) / (EC3) * 100

Determination of membrane injury index (MII) and membrane stability index (MSI)

Two sets, for each treatment, of 0.2 g of seedling shoots were weighed out and put in 20 mL distilled water. The first group was heated to 40°C for 30 min, while the second group was heated to 100°C for 15 min. The EC of each group was determined using EC meter. The Membrane Injury Index (MII) is calculated from this equation [22].

MII % = (EC_{40}/EC_{100}) *100

Membrane stability index was assessed by subtracting the value of MII from 100 [23].

2.2.2.4. Determination of proline

Proline was measured using Bates method [24] with some adjustments made by Khalil et al. [25]. About 0.5 g of dry leaves of each treatment were incubated with a reagent containing sulfosalicylic acid with an equivalent amount of glacial acetic acid and ninhydrin. The tubes were heated at 100 °C for 2 h. 5 ml toluene was added to extract the developed color after cooling down the sample. The absorbance was measured at 520 nm, and Standard proline dilutions were used to construct the standard curve.

2.2.2.5. Total phenolic content (TPC)

0.1 g dry tissue was incubated in 10 mL methanol (80%) for one week. After filtration, 50 µL of the extract and 1000 µL of 2% Na_2CO_3 were combined. After 5 min incubation at room temperature, 50 µL of 1 N Folin-Ciocalteu reagent was added, and the mixture was left for 30 min [26]. According to Zeitoun et al. [27]; The optical density was measured by using JENWAY 7305 (UK) at 720 nm, and then the total polyphenol content was represented as microgram gallic acid equivalents (GAE) per gram dry weight of the sample (µg GAE/g).

2.2.2.6. Total flavonoid content (TFC)

A mixture of 300 µL of methanol extract was added to 30 µL of 5% $NaNO_2$ then 60 µL $AlCl_3$ (10%) was added to the mixture after it had stood for 5 minutes at room temperature. Then, 200 µL of 1M NaOH was added to the mixture, and the absorbance was measured at 500 nm. The number of total flavonoids was represented in microgram quercetin equivalents (QE) per gram of dry sample (µg QE/g) were

used to quantify the overall flavonoid content [28].

2.3. Statistical Analysis:

Statistical analysis was performed on the data, and the COHORT/COSTAT software (798 Lighthouse Ave. PMB 329, Monterey, CA, 93940, USA) was used to compare means. The treatments were applied to subjects where ANOVA (analysis of variance) procedure could determine if the treatments had a significant effect on the observed values. The experimental design type that was chosen was the one-way totally randomized kind. Following the ANOVA, mean tests were used to compare the means within each treatment group to identify any significant differences. A mean test with a significance threshold of $P \leq 0.05$ was selected as the least significant difference (LSD) test. It should be noted that 10 plants were obtained for each character in the growth parameters, while three duplicate samples were examined for another parameter; nevertheless, the corresponding tables only provide the mean values.

3. Results

Three bacterial strains (P5, X1 and X3) showed the best capability in degrading naphthalene as a sole carbon source beside its plant growth promoting criteria.

The presence of *Bacillus lecheniformis* and *Bacillus aerius* led to a significant increase in short length, fresh and dry weight (**Table 2**). Both shoot water contents and leaf area did not show a significant difference between different treatments. The highest shoot length was recorded in T4 at 17.9 cm, while the lowest was observed in T1 at 13.3 cm. Similarly, shoot fresh weight was highest in T5 at 3.22 g, whereas T1 exhibited the lowest fresh weight (2.49 g).

Table 2. Effect of different bacterial strains on growth parameters of bean shoot at seedling stage (21-days old).

Treatments	Shoot length (cm/plant)	Shoot fresh wt. (g/plant)	Shoot dry wt. (g/plant)	Shoot water percentage %	Leaf area (Cm^2)
T1	13.3^{b}±0.61	2.49^{b}±0.13	0.22^{b}±0.01	91.09^{a}±0.77	0.06^{b}±0.02
T2	14.5^{b}±0.76	2.51^{b}±0.25	0.22^{b}±0.02	91.24^{a}±0.89	0.09^{ab}±0.004
T3	13.83^{b}±0.44	2.9^{ab}±0.09	0.23^{b}±0.01	91.95^{a}±0.51	0.11^{a}±0.01
T4	17.9^{a}±0.21	3.2^{a}±0.16	0.28^{a}±0.01	91.21^{a}±0.27	0.11^{a}±0.01
T5	17.66^{a}±0.17	3.22^{a}±0.16	0.25^{ab}±0.01	92.31^{a}±0.78	0.09^{ab}±0.004
LSD	1.6	0.5	0.04	2.15	0.03

The values listed represent the mean ± standard error (SE), (n= 3). Different superscript letters refer to significant variation in each column with the least significant difference (LSD) at $P \leq 0.05$. T1= control without bacteria, T2= control with *Rhizobium*, T3= *Pseudomonas MAP5* with *Rhizobium*, T4 = *Bacillus lecheniformis* with *Rhizobium*, T5= *Bacillus aerius* with *Rhizobium*)

Meanwhile, root length and fresh weights recorded a significant increase in response to bacterization with *Pseudomonas MAP5* and *B. lecheniformis* (**Table 3**). Notably, the highest root length was recorded in T3 at 21.5 cm, while the lowest was observed in T1 at 8.17 cm. Similarly, root fresh weight was highest in T4 at 2.12 g and lowest in T5 at 0.86 g. The water content of root did not show a significant difference. The results represented for physical growth parameters indicated that the studied strains made a significant difference in bean growth.

Table 3. Effect of different bacterial strains on growth parameters of bean root at seedling stage (21-days old).

Treatments	Root length (cm/plant)	Root fresh wt. (g/plant)	Root dry wt. (g/plant)	Root water percentage %
T1	8.17^{d}±0.66	1.19^{c}±0.03	0.14^{b}±0.01	88.16^{c}±0.21
T2	8.23^{d}±0.37	0.967^{cd}±0.03	0.07^{d}±0.003	93.05^{ab}±0.38
T3	21.5^{a}±0.58	1.76^{b}±0.13	0.10^{c}±0.01	94.16^{a}±0.09
T4	18.27^{b}±0.50	2.12^{a}±0.13	0.17^{a}±0.01	91.77^{b}±0.86
T5	10.17^{c}±0.44	0.86^{d}±0.04	0.07^{d}±0.01	92.15^{ab}±0.1
LSD	1.6	0.27	0.02	2

The values listed represent the mean ± standard error (SE), (n=3). Different superscript letters refer to significant variation in each column with the least significant difference (LSD) at $P \leq 0.05$. (T1= control without bacteria, T2= control with *Rhizobium*, T3= *Pseudomonas MAP5* with *Rhizobium*, T4 = *Bacillus lecheniformis* with *Rhizobium*, T5= *Bacillus aerius* with *Rhizobium*)

Chlorophyll a & b and subsequently total chlorophyll as well as carotenoids showed a significant increase in all treatments but T2 in which the main symbiont is alone (**Table 4**). The highest chlorophyll a content was recorded in T4 at 0.56 mg/g f. wt., whereas the lowest value was observed in T2 at 0.38 mg/g f. wt. Similarly, chlorophyll b content was highest in T4 (0.17 mg/g f. wt.) and lowest in T2 and T1 (0.13 mg/g f. wt.). Co-inoculation of plants with *B. lecheniformis* bacteria, along with other plant growth-promoting strains, increased the plants' chlorophyll and carotenoid content. This enhanced accumulation benefits plant photosynthetic processes and overall health.

Table 4. Effect of different bacterial strains on photosynthetic pigment of bean seedling stage

Treatments	Chl.a (mg/g f. wt.)	Chl.b (mg/g f. wt.)	Total chlorophyll (mg/g f. wt.)	Carotenoids (mg/g f. wt.)
T1	0.47^{a}±0.08	0.13^{a}±0.02	0.60^{ab}±0.08	0.15^{a}±0.02
T2	0.38^{a}±0.05	0.13^{a}±0.01	0.51^{b}±0.06	0.12^{a}±0.02
T3	0.46^{a}±0.04	0.145^{a}±0.01	0.61^{ab}±0.045	0.17^{a}±0.03
T4	0.56^{a}±0.05	0.17^{a}±0.01	0.73^{a}±0.06	0.19^{a}±0.02
T5	0.52^{a}±0.04	0.15^{a}±0.03	0.67^{ab}±0.03	0.18^{a}±0.03
LSD	0.17	0.05	0.18	0.08

The values listed represent the mean ± standard error (SE), (n= 3). Different superscript letters refer to significant variation in each column with the least significant difference (LSD) at $P \leq 0.05$. (T1= control without bacteria, T2= control with *Rhizobium*, T3= *Pseudomonas MAP5* with *Rhizobium*, T4 = *Bacillus lecheniformis* with *Rhizobium*, T5= *Bacillus aerius* with *Rhizobium*)

Interestingly, MII significantly decreased in response to different bacterization treatments, however, *Pseudomonas MAP5* showed the lowest value. In contrast, MSI recorded lower values with these treatments and electrolyte leakage decreased significantly particularly in *Bacillus aerius* bacterized plants (**Table 5**). T3 exhibiting the lowest MII value (18.78%) and T1 recording the highest (44.08%). In contrast, the Membrane Stability Index (MSI) increased significantly across treatments, with the highest value observed in T3 (81.22%) and the lowest in T1 (55.92%).

Table 5. Effect of different bacterial strains on membrane features (MII= Membrane Injury Index, MSI= Membrane Stability Index).

Treatments	MII (%)	MSI (%)	Electrolyte leakage (%)
T1	44.08^{a}±2.24	55.92^{d}±2.24	28.17^{a}±2.45
T2	27.76^{c}±1.67	72.24^{b}±1.67	24.31^{ab}±1.90
T3	18.78^{d}±0.49	81.22^{a}±0.49	20.67^{bc}±0.67
T4	32.68^{b}±1.43	67.32^{c}±1.43	21.22^{bc}±0.62
T5	35.75^{b}±0.95	64.25^{c}±0.95	16.71^{c}±0.89
LSD	4.7	4.7	4.7

The values listed represent the mean ± standard error (SE), (n= 3). Different superscript letters refer to significant variation in each column with the least significant difference (LSD) at $P \leq 0.05$. T1= control without bacteria, T2= control with *Rhizobium*, T3= *Pseudomonas MAP5* with *Rhizobium*, T4 = *Bacillus lecheniformis* with *Rhizobium*, T5= *Bacillus aerius* with *Rhizobium*.

Total phenolic contents showed a significant increase in response to all bacterial treatments; however, total flavonoids showed the most significant increase in response to *Bacillus lecheniformis* and proline content significantly decreased due to all bacterial treatments (**Table 6**). The highest phenolic accumulation was recorded in T2 at 12.18 mg GAE/g d. wt., while the lowest was observed in T1 at 8.95 mg GAE/g d. wt. Similarly, total flavonoid content exhibited the most significant increase in T4 at 4.40 mg Rutin/g d. wt., whereas the lowest value was recorded in T3 at 2.59 mg Rutin/g d. wt. The study results indicate that *Bacillus lecheniformis* bacterial inoculation enhances production of secondary metabolites while decreasing proline accumulation and leading to better stress tolerance and improved plant growth.

Table 6. Effect of different bacterial strains on phenolic, flavonoids content and proline.

Treatments	Total phenolic content (mg GAE/g d. wt.)	Total flavonoids content (mg Rutin/g d. wt.)	Proline (mg g^{-1}d. wt)
T1	8.95^{c}±0.23	3.43^{b}±0.05	1.43^{a}±0.03
T2	12.18^{a}±0.14	4.09^{ab}±0.14	1.14^{b}±0.06
T3	12.09^{a}±0.34	2.59^{c}±0.12	0.99^{bc}±0.08
T4	12.16^{a}±0.11	4.40^{a}±0.30	1.01^{bc}±0.04
T5	10.84^{b}±0.19	3.76^{ab}±0.34	0.90^{c}±0.04
LSD	0.68	0.69	0.16

The values listed represent the mean ± standard error (SE), (n= 3). Different superscript letters refer to significant variation in each column with the least significant difference (LSD) at $P \leq 0.05$. T1= control without bacteria, T2= control with *Rhizobium*, T3= *Pseudomonas MAP5* with *Rhizobium*, T4 = *Bacillus lecheniformis* with *Rhizobium*, T5= *Bacillus aerius* with *Rhizobium*.

The ANOVA test was conducted to determine if the results of the proposed treatment (control) and the other treatments have a significant difference or not; P-value < 0.05 will demonstrate significant superiority. By contrast, a P-value > 0.05 shows that the results have no significant difference (**Table 7**). The low P-value of 0.0001, significantly smaller than the commonly accepted significance level of 0.05, provides strong statistical evidence that the growth parameters of the different treatments are indeed significantly different from each other.

Table 7. Analysis of variance (ANOVA) of the feature selection results for morphology

ANOVA Table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (betweencolumn)	56.74	4	14.18	F (4, 10) = 19.54	P=0.0001
Residual (within columns)	7.26	10	0.726		
Total	64	14			

SS denotes (the sum of squares), DF (degrees of freedom), DFn denotes the DF numerator, DFd denotes the DF denominator and MS (mean square).

The ANOVA test for pigments (**Table 8**) indicated strong statistical evidence that the pigment contents of the different treatments are indeed significantly different from each other. A P-value of 0.0001 indicates that these differences are highly significant, as they are much smaller than 0.05.

Table 8. Analysis of variance (ANOVA) of the feature selection results for pigments

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.005453	4	0.001363	F (4, 10) = 19.54	P=0.0001
Residual (within columns)	0.0006978	10	6.98E-05		
Total	0.006151	14			

SS denotes (the sum of squares), DF (degrees of freedom), DFn denotes the DF numerator, and DFd denotes the DF denominator and MS (mean square).

There are significant differences in the impact of different treatments on proline contents (**Table 9**). A P-value of 0.0003 indicates that these differences are highly significant.

Table 9. Analysis of variance (ANOVA) of the feature selection results for proline

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.1544	4	0.03859	F (4, 10) = 15.46	P=0.0003
Residual (within columns)	0.02496	10	0.002496		
Total	0.1793	14			

SS denotes (the sum of squares), DF (degrees of freedom), DFn denotes the DF numerator, DFd denotes the DF denominator, and MS (mean square).

ANOVA analysis in **Table (10)** showed low P-value that indicates the results of phenols vary significantly between treatments, demonstrating the efficacy of the applied treatments in influencing phenol-related outcomes.

Table 10: Analysis of variance (ANOVA) of the feature selection results for phenols. In this table, SS denotes (the sum of squares), DF (degrees of freedom), DFn denotes the DF numerator, and DFd denotes the DF denominator. MS (mean square).

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.06626	4	0.01657	F (4, 10) = 41.97	P<0.0001
Residual (within columns)	0.003947	10	0.000395		
Total	0.07021	14			

P-value of ANOVA analysis in **Table (11)** indicates that there is a statistically significant difference between treatments in terms of flavonoids.

Table 11. Analysis of variance (ANOVA) of the feature selection results for flavonoids

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	1.699	4	0.4248	F (4, 10) = 10.17	P=0.0015
Residual (within columns)	0.4176	10	0.04176		
Total	2.117	14			

SS denotes (the sum of squares), DF (degrees of freedom), DFn denotes the DF numerator, DFd denotes the DF denominator, and MS (mean square). The residual plots (Figure 1A and B) suggest variability in the discrepancy between the actual and theoretical means across treatments. The substantial effect size (R-squared) further indicates that the treatments significantly contribute to the observed variation in the data. This is consistent with the patterns observed in the QQ plot (Figure 1C).

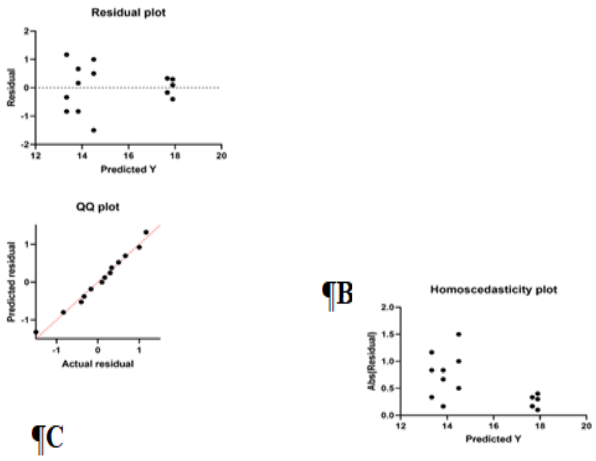

Figure 1. Visualizing the ANOVA test applied to the proposed feature selection method, results showing significance between treatments. A) Residual plot, and B) Homoscedasticity plot, C) QQ plot.

4. Discussion and conclusion:

Plant growth-promoting bacteria (PGPB) are a group of beneficial bacteria that can enhance plant growth and development by various mechanisms including direct and indirect ones. The use of PGPB as bioinoculants has gained considerable interest particularly for sustainable agriculture, the need to coop the increasing demand for food production in an environmentally- friendly way.

Vicia faba naturally forms a symbiotic relationship with *Rhizobium*, but this does not eliminate the need for plant growth-promoting bacteria (PGPB) to further enhance its growth. Several studies showed the potential effect of various PGPB along with *Rhizobium* particularly *Pseudomonas* [29] and Bacillus [30] on bean growth and yield.

In this study, the used strains were isolated from the nodules of *Phaseolus vulgaris* (*Pseudomonas MAP5)* [18] and the *Calligonum* associated *Xanthoria parietina* (*Bacillus lecheniformis and Bacillus aerius*) [19]. These strains showed positive results toward the plant growth promoting activities particularly IAA and GA_3 production and this might explain the progressive increase in bean shoot and root length and fresh weight [31]. Not only as a growth promoter but also *Pseudomonas* different species are used as bio control agents [31,32]. *Bacillus lecheniformis* [33] and *Bacillus aerius* [34] are also employed for controlling phyto-pathogens. Both *Pseudomonas* and *Bacillus* are used as bio-degraders of polyaromatic hydrocarbons [35,36].

Pseudomonas were reported to improve bean growth and yield either alone or in combination with [37]. It also was reported to alleviate salinity stress conditions on plants [38]. *Bacillus* was also reported to improve

bean yield [29] and to alleviate salinity stress upon [39].

In this study, both *Bacillus* strains were able to increase significantly the amounts of chlorophylls as well as total chlorophylls and carotenoids, a result that has been reported previously [30,29]. Generally, the enhancement in N, P and K uptake significantly increases chlorophyll contents. For both *Bacillus* strains used in this study, they are both efficient phosphate solubilizers and they were also able to grow in nitrogen free media (data not shown) and this might be the reason for increasing chlorophyll contents.

The decrease in electrolyte leakage was reported in response to *Bacillus* [40] in soyabean as a sign for its ability to alleviate salt stress. The same results were obtained in *Vigna radiata* received *Pseudomonas* bacterization under drought stress [41].

The amounts of phenolic compounds in this study increased significantly in all bacterized plants compared to the control plants. Both phenols and flavonoids of strawberry increased significantly in response to Pedobacter sp. CC1 [42]. In our study, *Bacillus lecheniformis* (T4 treatment) led to a significant increase in flavonoid contents. Proline contents increased in tobacco in response to Bacillus megaterium OSR-3 [43] a result that is not compatible with our results.

Proline functions as an osmoprotectant and stress marker through various stress conditions, allowing the cells to decrease oxidative damage while stabilizing proteins and maintaining osmotic balance. The reduction in proline content detected in plants treated with *Bacillus lecheniformis* showed signs of lower physiological stress levels when compared to untreated plants. Proline accumulation is mainly an environmental stress adaptation mechanism since plants use proline to protect themselves in unfavorable conditions. Plants tend to suppress proline biosynthesis processes during favorable growth conditions since excess proline production's high energy costs exceed their requirements. Numerous studies demonstrate how plant growth-promoting rhizobacteria (PGPR) eliminate stress factors, decreasing the requirement of stress metabolites, including proline [40 & 41]. he membrane stability index increased, and the membrane injury index decreased in bacterized plants, providing additional confirmation for this explanation. Plants with improved membrane stability demonstrated better capacity to handle exterior stressors, which reduced the need to produce proline. The *Bacillus lecheniformis* treatment raises plant phenolic and flavonoid content, which indicates this microorganism effectively controls secondary metabolism, so plants use fewer antioxidants; hence, their proline synthesis requirement decreases.

Bacillus lecheniformis demonstrates its ability to enhance plant resistance by improving both nutrient assimilation and hormonal output of auxins and gibberellins, which in turn results in increased plant vigor alongside stress tolerance [29 &30]. A well-regulated environment through increased nutrients and hormones creates conditions that direct plant resources toward development rather than producing proline to combat stress.

The dissimilar findings between tobacco (Nicotiana tabacum) and Bacillus megaterium OSR-3 results can be explained due to different bacterial strain responsiveness, host plant reactions, and experimental setup dissimilarity. Future investigations exploring specific bacterial-induced molecular and biochemical mechanisms in proline metabolism and plant stress response patterns under different environmental conditions would deepen our knowledge of bacterial strain effects. The modulation of secondary metabolites contents in our study indicated the ability of the used bacterial strains to stimulate defense response mechanisms in bean plants, a strategy that would alleviate the effect of both biotic and abiotic stress on plants. The strains used in this study were nominated based on their ability to use and degrade naphthalene as a sole carbon source. The growth enhancement observed in this study paved the way for using these strains as poly aromatic hydrocarbons degraders to alleviate their negative effect on plant yield.

This research discusses a new agricultural system that combines bioremediation plant growth-promoting bacteria (PGPB) to improve *Vicia faba* growth and metabolic levels. Applying *Pseudomonas MAP5 Bacillus*

lecheniformis and *Bacillus aerius* presents substantial progress for agriculture production and environmental recovery through their naphthalene degradation capability using it as their sole carbon source. The evaluation yielded significant findings about bacterial strains that function as dual-use agents to enhance plant growth and reduce the impacts of polycyclic aromatic hydrocarbons on contaminated soils while boosting photosynthetic pigment formation and secondary metabolite synthesis.

This study supports sustainability by developing microbial interactions that replace chemical pesticides, allowing soil health enhancement and reducing environmental contamination. The bacterial strains show dual capability for protecting plant membranes while reducing electrolyte leakage and boosting phenolic and flavonoid content, as well as stress tolerance under changing environmental conditions and decreasing soil fertility.

Future investigations must apply these bacterial inoculants across agricultural fields to validate their performance in various environmental settings. Researchers need to investigate microbial synergies and develop optimal delivery strategies for these bacteria to enable their implementation in sustainable farming systems across crops. The examined data demonstrates a significant potential for implementing microbial biotechnology methods as they establish an essential link between crop production levels and ecological sustainability.

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