Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Evaluation of various carrier material compositions for phosphorus availability, growth, and survival of PGPR consortia

Qais Ali Lund
Sindh Agriculture University
Qurban Ali Panhwar
Nuclear Institute of Agriculture
Muhammad Saleem Sarki
sarkisaleem@yahoo.com

Sindh Agriculture University
Amanat Ali
Nuclear Institute of Agriculture
Allah Wadhayo Gandahi
Sindh Agriculture University
Zohaib ur Rehman Bughio
Sindh Agriculture University
Kashaf Mari
University of Karachi

Research Article

Keywords: Bacterial population, Carrier material, Consortia, Incubation, Phosphate sources, Solubilization

Posted Date: November 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11408315/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Evaluation of various carrier material compositions for phosphorus availability, growth, and survival of PGPR consortia

Qais Ali Lund,

Department of Soil Science, Sindh Agriculture University, Tandojam, Sindh, Pakistan

ORCID: 0009-0006-9441-7713

Qurban Ali Panhwar

Soil and Environmental Sciences Division, Nuclear Institute of Agriculture, Tandojam, Sindh, Pakistan

ORCID: 0000-0001-5117-3890

Muhammad Saleem Sarki*

Department of Soil Science, Sindh Agriculture University, Tandojam, Sindh, Pakistan

ORCID: 0009-0007-7281-837X

Amanat Ali

Soil and Environmental Sciences Division, Nuclear Institute of Agriculture, Tandojam, Sindh, Pakistan

ORCID: 0000-0002-0479-6304

Allah Wadhayo Gandahi

Department of Soil Science, Sindh Agriculture University, Tandojam, Sindh, Pakistan

ORCID: 0009-0007-0491-6218

Zohaib Ur Rehman Bughio

Agriculture Extension Wing, Agriculture Supply and Prices Department Sindh, Pakistan

ORCID: 0000-0001-6248-2664

Kashaf Mari

Department of Microbiology University of Karachi, Sindh, Pakistan

ORCID: 0009-0009-2315-4874

Evaluation of various carrier material compositions for phosphorus availability, growth, and survival of PGPR consortia

Qais Ali Lund[1], Qurban Ali Panhwar[2], Muhammad Saleem Sarki[1*], Amanat Ali[2] Allah Wadhayo Gandahi[1], Zohaib ur Rehman Bughio[1,3] and Kashaf Mari[4]

[1]*Department of Soil Science, Sindh Agriculture University, Tandojam, Sindh, Pakistan*

[2]*Soil and Environmental Sciences Division, Nuclear Institute of Agriculture, Tandojam, Sindh, Pakistan*

[3]*Agriculture Extension Wing, Agriculture Supply and Prices Department Sindh, Pakistan*

[4]*Department of Microbiology University of Karachi, Sindh, Pakistan*

[*]*Corresponding author: sarkisaleem@yahoo.com*

ABSTRACT

Plant-growth-promoting rhizobacteria (PGPR) can enhance plant growth and crop productivity with soil nutrient improvement. This study aimed to screen and characterizes PGPR to evaluate their ability to solubilize insoluble phosphorus (P) from various sources of phosphates. The selected four PGPR (NIA-NB1, NIA-NB3, NIA-NB6, and NIA-NB8) strains were tested to evaluate the P-solubilizing efficiency under *in vitro* conditions. These strains were combined into two different consortia, each with two different phosphate sources. Both PGPR (NBRIP and RP) phosphate sources initially showed a consistent increase in bacterial population (0.0-9.62 Log CFU mL^{-1}) but after 24 hr RP showed a decline during incubation. Both consortia solubilized phosphate more effectively using NBRIP (21.25-46.28%) compared to RP (4.69-5.29%). Three carrier materials were tested for their impact on PGPR growth, survival, and phosphate solubilization. All carrier materials showed a slight decrease in pH and an increase in organic matter contents during incubation. All carriers were capable of phosphate solubilization, with the highest solubilization (8.73 %) achieved by the CM3+RP3 carrier material. All the PGPR carrier materials all carriers supporting PGPR survival after inoculation and showing growth throughout the incubation period. The highest population recorded in the CM2+RP3 carrier (9.29 Log $CFUg^{-1}$) after 90 days of incubation. Thus, using PGPR with various carrier materials and insoluble phosphate sources demonstrates the potential to reduce production costs and minimize environmental contamination by reducing reliance on chemical fertilizers.

Keywords: Bacterial population, Carrier material, Consortia, Incubation, Phosphate sources, Solubilization.

1- INTRODUCTION

Phosphorus (P) is an important nutrient for the growth of plants since it is a structural element of important biomolecules and participates in basic cellular metabolism (Santoro et al., 2024). It plays a role in respiration and photosynthesis, making it crucial for growth, development, and reproduction (Jiang et al., 2019). Phosphorus is absorbed by plants in the forms of ($H_2PO_4^-$/HPO_4^{-2}) primary orthophosphate and secondary orthophosphate (Prathap et al., 2022). Due to the limited amount of soil P accessible, modern agriculture primarily relies on P fertilizer. To meet the demands of food security and agricultural sustainability, however, increasing the P use efficiency (PUE) of crops has become crucial due to the low efficiency of applied P absorbed by plants and the limited nature of P fertilizer (Heuer et al., 2017). After all, it is involved in enzymatic reactions and cell division because it is a constituent of nucleoproteins involved in reproduction. It could be said that P is involved in cell reproduction, which would affect plant yield (Liu et al., 2017).

Pakistan lies in the arid to semi-arid region of the earth, with latitudes between 24 N°- 37 N° and longitudes between 61E° -77E° (Hussain et al., 2010). The majority of dry regions soil with low moisture content have P deficiencies (Bhattacharya and Bhattacharya, 2021). Pakistani soils are predominantly alkaline (pH > 7.0) and calcareous (CaCO3> 3.0%) in nature (Barrow et al., 2015). P availability is influenced by a number of variables, including calcium and iron compounds, microbial presence or absence, soil pH, texture, and structure (Gerard, 2016). Soils with dominant Fe+3 and Al+3 at low pH (<5 or acidic) or by Ca+3 in alkaline soils pH >7, resulting low mobility and availability of phosphorus in the soil (Prathap et al., 2022). About 90% of Pakistani soils are deficient in P (Kaya et al., 2020). According to reports, Pakistan's fertilizer use in 2021 was 157.3 kg ha-1, growing at an average yearly rate of 5.36% faster than previously estimated (Narractives et al., 2023). While constant use of chemical fertilizer for greater yield would increase the production cost simultaneously environmental pollution and degrade the soil quality (Kavitha & Rajini et al., 2013). Thus, bio-fertilizer is an eco-friendly & sustainable agriculture approach (Packialakshmi & Alia, 2014).

The bio-fertilizers contain effective microorganisms that increase plant growth and development by providing them with nutrients (Sivasakthivelan & Saranraj, 2013). A bio-fertilizer consists of living microorganisms such as bacteria. When it is applied to soil, the

inoculated microorganisms colonize the rhizosphere of the plant, and after that they increase the supply of nutrients to the host plant (Youssef & Eissa, 2014).

It has been reported that plant growth-promoting rhizobacteria (PGPR) increase plant growth by producing gibberellic acid, IAA (indole acetic acid), siderophore, atmospheric nitrogen fixation, and inorganic phosphate solubilization (Naher et al., 2013; Panhwar et al., 2014). PGPR helps to prevent such situations that can limit plant growth, which is known as indirect growth promotion (Glick et al., 1999). On the other hand, PGPR's direct plant growth promotion consists of giving the plant nutrients or helping it absorb them. Ethylene, auxin, gibberellins, and cytokinin are among the plant hormones that are most likely produced by the PGPR (Afzal et al., 2014). By increasing the amount of mineral nutrients that are available and regulating the levels of phytohormones (cytokinins, auxins, and gibberellins), which affect different stages of plant growth, the PGPRs stimulate plant growth through interactions between plant roots (Kumar et al., 2018).

The phosphate uptake may potentially be aided by interactions with beneficial microbes, brought on by P shortfall site (Oldroyd & Leyser, 2020). The Phosphate solubilizing bacteria (PSB) are the main group of potential microbes for the execution of P in different crops. P inoculated material or biofertilizer has some bacterial species like *Pseudomonas, Bacillus,* and fungal species *Aspergillus Mycobacterium, Micrococcus, Flavobacterium, Rhizobium, Mesorhizobium*, and *Sinorhizobium* (Sharma et al., 2013; Mondal et al., 2017). The PSB species have the ability to turn insoluble form PO_4^- constituents into plants available for better uptake (Baliah & Priya et al., 2017). Moreover, with the help of PSB phosphate could be obtained by many ways such as: soil acidification, elemental chelation, exchange reactions and organic acid production in soil (Packialakshmi & Alia et al., 2014, Adnan et al., 2017). The PSBs are widely distributed and vary in number and morphology depending on the soil. The physical and chemical characteristics of the soil, the amount of organic matter, the P concentration, and cultural activities all affect the PSB population (Kim et al., 1998). Hence, keeping in view the above mentioned facts, the current study was carried out to characterize and screen the PGPR for P-solubilization efficiency from various P-sources and to evaluate the suitable carrier material for growth and survival of PGPR and P-solubilization.

2. MATERIALS AND METHODS

The research study was carried out at Soil Microbiology Laboratory, Nuclear Institute of Agriculture (NIA), Tandojam and Department of Soil Science Sindh Agriculture University, Tandojam. The selected four PGPR strains using two consortia (Consortium-1: NIA-NB1 and NIA-NB3, Consortium-II: NIA-NB6, and NIA-NB8) were evaluated utilizing two insoluble phosphatic sources for phosphate quantification. In addition, a carrier material including with phosphate insoluble (rock phosphate) source was evaluated for the bacterial survival and phosphate solubilization.

(A) Determination of potential plant growth promoting rhizobacteria for phosphate solubilization efficiency using various insoluble phosphatic sources

Quantification of phosphate solubilization

The potential PGPR were selected from the qualitative phosphatic solubilization efficiency and other plant beneficial traits. The selected two PGPR consortia (Conso-I & Conso-II) were used for quantitative phosphate solubilization in two different modified P media (NBRIP & RP) during the incubation study. The culture was collected after 24 hours interval and bacterial viable count, optical density, pH, electrical conductivity and available phosphorus were recorded.

Inoculum preparation

The best potential selected PGPR strains from previous *in vitro* study were used in developing bacterial consortium. The starter pure culture of PGPR was grown in nutrient broth for 48 h and then inoculated in different modified media with different insoluble P forms (NBRIP & RP). The culture was shaken using the mechanical shaker for 96 hours and optical density was recorded during the incubation period.

Preparation of broth cultures

Preparation of NBRIP broth media

The tri-calcium phosphate was added as a source for phosphorus in NBRIP broth (Nautiyal, 1999). The NBRIP broth contains (g L^{-1}): $MgCl_2.6H_2O$ 5 g, $MgSO_4$. H_2O 0.25 g, KCl 0.2 g, $(NH_4)_2SO_4$ 0.1 g, $Ca_3(PO_4)_2$ 5 g, amended with glucose 10 g.

Preparation of RP broth media

The rock phosphate (RP) broth media was modified from NBRIP and supplemented with 5 g L^{-1} of rock phosphate (RP) instead of tri-calcium phosphate.

Both of the broths were inoculated with the respective (5×10^9 Log_{10} CFU mL^{-1}) of conso-I and conso-II inoculums. Flasks containing 200 mL of inoculated media were incubated at 30 °C on a Kottermann 4020 shaker at a medium speed of 80 rpm.

Total bacterial counts in PGPR Consortia

The total bacterial counts in the consortia were determined after 0, 24, 48, 72, and 96 hours of incubation during the culture growth. After serial dilution, the total bacterial counts were determined following the spread plates counting method on nutrient agar (NA) plates (Somasegaran and Hoben, 1985).

Electrical Conductivity and pH decline of PGPR Consortia culture

The Electrical Conductivity (EC) of the carriers was analyzed by EC meter using (1:5) carrier water extract and the pH by using standard pH meter at (1:5) carrier water extract (Benton 2001), after the 0, 24, 48, 72 and 96 hours of the incubation period.

Quantification of available phosphorus in broth cultures

For available P measurement, 2 mL of samples were taken at each sampling time during the incubation period. After 15 minutes of sedimentation, the samples were centrifuged for 5 minutes at 4000 x g. After passing through 0.2 µm filter paper, the supernatant was stored at -200 C until it was time for analysis. Available P was calculated using Murphy and Riley's (1962) methodology.

(B) Evaluation of various carrier material compositions for phosphorus availability, growth, and survival of PGPR consortia

Finally, the selected potential PGPR consortium was inoculated in different carrier materials. That contained rock phosphate (RP) hardly soluble source of P. Total bacterial count, pH, OM content, P, and K were determined during the study with 30 days of interval. The incubation study was continued till 90 days after inoculation.

Preparation of carriers and inoculation of PGPR consortium

The carrier materials i.e., compost including various organic materials were packed in the autoclave able bags, moistened 40% using distilled water and sterilized at the 121^0C for 60 minutes in the autoclave. After the temperature lowers, PGPR consortium was injected using sterilized syringe in each bag. The inoculated bags were then kept in the laboratory for incubation.

Phosphorus, potash, and organic matter contents in carrier materials

The phosphorus and potash in the carriers were analyzed after inoculation of PGPR consortium. The available phosphorus (P) and potash (K) were analyzed through AB-DTPA

(Soultanpur and Schwab 1977) method. The organic matter content of carriers was analyzed by (Walkely and Black 1934) method while the organic carbon was assessed using the formula (OC% = OM/1.724 × 100) (Walkely & Black, 1934) before inoculation of PSB.

Total bacterial count in the carrier after PGPR inoculation

The total bacterial count in the carrier was determined after 30, 60, 90 and 120 days of inoculation. A 5g of carrier sample was transferred to a 100 mL conical flask containing 45 mL of sterilized distilled water. The mixture was shaken dynamically on a rotatory shaker for 15 minutes to suspend bacterial cells. After serial dilution, the total bacterial counts were analyzed following spread plates counting method on nutrient agar (NA) media plates (Somasegaran and Hoben, 1985).

Determination of pH in various carrier materials

The pH of the carrier materials was analyzed using by standard pH meter at (1:5) carrier water extract (Benton 2001), after the 30, 60, and 90 days of the inoculation.

Statistical analysis

Using the STATISTIX 8.1 computer program, the gathered data will be statistically examined (Satatix, 2006). The LSD test, which stands for least significant difference, was used where needed to compare the differences between the treatments.

3. RESULTS

(A) Quantification of P from various P sources by potential screened PGPR consortia

Electrical conductivity (EC) and pH in NBRIP as P- source inoculated with PGPR Consortia

The electrical conductivity (EC) gradually increased until 72 hours, then decreased. The pH in the control increased over time when no microbes were added and in the inoculated media with Conso-1 using modified NBRIP media (Table 1). The lowest EC was recorded initially before the incubation period, and the lowest pH was recorded after 96 hours of incubation in NBRIP media with Conso-1. There was a slight increase in the culture medium's EC (3.3-3.8 dS m^{-1}), while minor changes were recorded in the control during the incubation period. The pH experienced a sudden drop during the incubation period, with the lowest pH (4.9) recorded after 96 hours and the highest recorded in the control during the incubation period.

In Conso-II the lowest electrical conductivity (EC) was observed in the control group without inoculation. In the control group, the highest EC was recorded after 72 hours

and then decreased. The lowest EC was recorded after 48 hours, while the highest was recorded at the time of inoculation. The highest pH was observed after 72 hours in the control group, while the lowest was observed in the sample preparation. Following the inoculation of Conso-II, the pH gradually decreased from 7.0 when the sample was prepared to 4.7 after 96 hours (Table 1).

Electrical conductivity (EC) and pH in RP as P-source inoculated with PGPR Consortia

The electrical conductivity (EC) was affected with the incubation time in various PGPR consortia. The lowest EC in the control treatment at the time of sample preparation, which gradually increased to (2.3 dSm^{-1}) after 48 hours. When inoculated with Conso-I the EC has increased to (3.45 dSm^{-1}) after 24 hours which can be said as the highest, later it decreases gradually till 96 hours. In terms of pH in the control group, pH gradually increased from (7.0 to 7.3) at 96 hours of incubation period, but in inoculated media, it drastically decreased to (4.2) at the lowest and highest (7.0) at the time of incubation (Table 2).

The EC and pH in a liquid culture containing RP and inoculated with Conso-II. After 24 hours of incubation, the highest EC (2.4 dSm^{-1}) gradually decreased until 96 hours of incubation (Table 2). In Conso-II containing samples, the lowest EC was recorded in the control at the time of inoculation, which increased to 3.32 dS m^{-1} after 48 hours of incubation as the highest level. In pH, the control group shows lowest pH (7.0). The pH in inoculated media decreased as lowest (4.1) in inoculated the pH is decreased astonishingly. And the highest at the time of sample preparation which was (7.0).

Determination of total bacterial count in various phosphatic sources in different consortia

The total bacterial counts were varied in both phosphatic sources in both PGPR consortia (Conso-I & Conso-II) during the incubation period. Total bacterial counts were recorded after inoculation with Conso-I containing Tri-calcium phosphate (NBRIP) as a source of P, the bacterial counts had increasing trends after the increase in incubation period. The highest bacterial count (9.62 CFU mL^{-1}) was observed after 96 hours of incubation, sudden decrease in bacterial counts seen after 24 hours of incubation and there were no bacteria before inoculation (Figure 1a). In Conso-II in NBRIP media the total bacterial population was astonishingly increased after 24 hrs of inoculation and suddenly dropped with the increase in incubation period particularly after 48 hrs of incubation period. The highest bacterial population (9.04 CFU mL^{-1}) was seen in 48^{th} hour of sampling, sudden decrease in population was seen after 72 hours of incubation and no population was seen before inoculation (Figure 1b).

In case of rock phosphate (RP) containing P source inoculated with Conso-I, no population was seen before inoculation and initially it was significantly increased till 24 hours of incubation. The highest count (7.53 CFU mL^{-1}) were noted after 24 hours of incubation then it was showing decline and minimum counts (5.05 CFU mL^{-1}) were recorded after 96 hours of incubation (Figure 4.3.6c). The figure shows that the total bacterial population in liquid culture when containing RP as P source and inoculated with Conso-II, no population was seen before inoculation and it was significantly increased with incubation time. The highest population (7.43 CFU mL^{-1}) was observed after 24^{th} hour of inoculation and after 72 hrs of incubation the population (4.93 CFU mL^{-1}) was decreased after 96^{th} hours of incubation period (Figure 1d).

Optical Density of NBRIP incubated with (a) Conso-I and (b) Conso-II

The determination of Optical Density (OD) in liquid culture containing tri-calcium phosphate as a source of P, inoculated with Conso-I. The optical density has increased with the increase in incubation period. The lowest OD (0.08 Cells mL^{-1}) was recorded in non-inoculated samples, and the highest (1.8 Cells mL^{-1}) were recorded after 96^{th} hour of inoculation. The determination of OD in liquid culture containing tri-calcium phosphate as a source of P, inoculated with Conso-II. The OD has increased (1.8 Cells mL^{-1}) rapidly at 24 hrs of incubation period and it was showing consistency in cells growth. The lowest OD was recorded in 48^{th} and 96^{th} hour of incubation period (Figure 2a, b).

Optical Density (OD) in RP as a hardly available source of P was recorded during incubation after inoculation with conso-I and it had an increasing (1.4 cells mL^{-1}) after 24 hours of incubation and afterward, it had a decreasing trend till 96 hours of the incubation period. The minimum OD (0.85 cells mL^{-1}) was recorded after 96 hours of incubation period. A similar trend was noticed in case second case of RP inoculated with conso-II. The maximum OD was recorded after 24 hours of incubation (1.9 cells mL^{-1}) and afterward, it was declining. The least OD (1.01 cells mL^{-1}) was observed after 96 hours of incubation period (Figure 2 c, d).

P-Solubilization efficiency (%) in NBRIP and RP media inoculated with Conso I & II

The P-solubilization is gradually increasing with the increasing incubation period both in NBRIP and RP inoculated with Conso-I and II during the incubation period. The NBRIP media had higher P-solubilization efficiency as compared to RP. Both Conso-I and II had an increasing trend of P-solubilization during the incubation period in both media. However, the highest P-solubilization (46.28**%)** was observed in Conso-I followed by

41.52% in Conso-II in NBRIP media after 96 hours of incubation. Whereas, the lower solubilization was observed in both Conso I and II inoculated in RP media. The highest (5.29%) P solubilization was recorded in RP inoculated with Conso-I after 96 hours of incubation period. Comparatively, lowest readings were found in Conso-II as compare to Conso-I during the incubation study (Table 3).

Kinetics of P solubilization by bacterial Consortia in different phosphatic broths

The solubilization rate (k) for different times during the incubation period was determined by using first order kinetics. By regression analysis it was found that the kinetics of P solubilization was best fitted in power model for both NBRIP and RP broths. The bacterial Conso mediated $Ca_3(PO_4)_2$ solubilization in NBRIP broth followed one group of model (Figure 3a, b). The steep of the curve (k) obtained by both Conso (Conso-I & Conso-II) followed power model and most of the P solubilized in culture solution within 96 h of incubation period, while Conso-II slight gradually solubilized P. In RP broth both bacterial Conso (Conso-I & Conso-II) solubilized P gradually and followed power model, whereas conso-II solubilized P faster than conso-I in this broth culture during the incubation period (Figure. 3c, d).

(B) Evaluation of suitable carrier material for growth and survival of PGPR and P-solubilization

From the laboratory study 2 finally conso-I was selected for its best characteristics and inoculated in different carrier materials (CM1, CM2 & CM3) including rock phosphate (RP) as P source. The incubation study was continued till 90 days after inoculation and data was recorded monthly.

Changes of pH in various carrier materials

The variation in the pH of various carrier materials (CM) was observed during the incubation period. After 30 days of PGPR consortium inoculation the pH of the different carrier materials had an increasing trend (7.9-8.3) and afterwards it was slightly decreasing with the incubation time. After 60 days of inoculation the pH range was from 7.7 to 7.9 and at 90 days of inoculation it was further decreased and was showing ranges from 7.3 to 7.8. After 90 days of inoculation the lowest pH was recorded in CM3+RP1 and CM3+RP2 (7.3) and maximum pH (7.8) was in CM3+RP0 (Table 4).

Changes of organic matter contents in various carrier materials

The various carrier materials were contained different organic matter contents and it was showing increase among the treatments during the incubation period. In most cases, the organic matter contents were increasing during the incubation period. Comparatively, the higher contents were found in PGPR inoculation with RP applied treatments. After 30 days the maximum contents (45.72%) were recorded in CM2+RP2 treatment. However, after 60 and 90 days of inoculation the highest organic matter contents (47.72% and 50.48%) were found in CM3+RP2 treatment (Table 5).

Changes of phosphate contents in various carrier materials (CM)

The available phosphate contents in various carrier material were dissimilar among the treatments during the incubation period. The addition of RP with bacterial consortium increased the phosphate contents during the incubation. However, the higher applied RP in the carrier materials treatments resulted in higher contents of phosphate during the incubation period. The highest contents 3.40%, 5.34% and 8.73% were observed in CM3+RP3 treatments during the incubation period at 30, 60, and 90 days respectively (Table 6).

Changes of potassium contents in various carrier materials

The potassium contents also varied among the various treatments during the incubation study. An increasing trend in potash contents was noted during the incubation period. Comparatively, the higher contents were recorded with the addition of RP with PGPR consortium in carrier materials. The highest contents (8.87%) were recorded in CM2+RP3 followed, by the CM3+RP3 treatment (8.83%) after 30 days of incubation period. However, after 60 days and 90 of incubation maximum (9.73 and 11.43 %) contents were observed in CM3+RP3 treatment respectively (Table 7).

Changes of total bacterial counts in various carrier materials

Total bacterial counts in various treatments were found among the treatment during the incubation period. An increasing trend was observed among the treatments during the incubation period. After 30 days of incubation the maximum bacterial counts (8.83 Log CFU g^{-1}) was found in CM2+RP2. However, after 60 days of incubation the highest bacterial counts (8.98 Log CFU g^{-1}) was found in CM1+RP3 followed by (8.96 Log CFU g^{-1}) in CM2+RP2. In case of 90 days after inoculation the maximum population (9.29 Log $_{10}$ CFU g^{-1}) was observed in CM2+RP3 followed by 9.28 Log $_{10}$ CFU g^{-1} CM3+RP2 (Figure 4).

DISCUSSION

The selected beneficial bacteria, used in both consortia (Conso-I and Conso-II) with different phosphorus (P) sources, demonstrated their ability to solubilize phosphorus. Combined bacterial strains performed better than individual strains, with synergistic combinations consistently enhancing performance. The most effective strain combinations were further evaluated through laboratory, greenhouse, and field studies. The results indicated that co-inoculation of rhizobacterial strains led to better growth promotion compared to single-strain inoculation (Khan et al., 2022a).

Initially, the pH of the broth culture decreased over the incubation period. The microorganisms tested were able to convert insoluble phosphorus into soluble phosphorus by lowering the pH of the culture medium. Phosphorus-solubilizing microbes are a reliable strategy for enhancing plant growth without causing environmental pollution (Alori et al., 2017). The microbes in the various consortia continued to solubilize phosphorus during incubation, creating acidic conditions in the culture medium. Specifically, these bacterial consortia solubilized phosphorus either by forming halo zones (23.62-67.41%) on media plates or from hardly soluble phosphorus sources (21.25-46.28%) in liquid culture under *in vitro* conditions.

Many soil microorganisms (PSB) are capable of solubilizing or mineralizing insoluble soil phosphates to release soluble phosphorus for plant uptake. The mechanism of inorganic phosphorus solubilization is explained by several theories. Commonly, this process involves the production of mineral-dissolving compounds such as organic acids, siderophores, protons, and hydroxyl ions (Alori et al., 2017). Additionally, phosphorus solubilization is generally attributed to the production of microbial metabolites, including organic acids, which lower the pH of the culture media (Timofeeva et al., 2022).

The potential Plant Growth-Promoting Rhizobacteria (PGPR) Consortium-I was selected for inoculation in various carrier materials (CM1, CM2, and CM3) with rock phosphate (RP) as the phosphorus (P) source. The incubation study continued for 90 days after inoculation. Initially, the pH of the carrier materials was alkaline (7.9-8.3) after 30 days of inoculation. Over the incubation period, the pH of the carrier materials decreased, with almost all treatments showing a pH below 8.0 after 90 days. Previous studies have reported similar results, attributing the pH decrease to the decomposition process and the production of organic acids following bacterial inoculation (Khan et al., 2022b).

The carrier materials used in this study contained substantial amounts of organic matter. Following PGPR consortium inoculation, the organic matter content increased over the incubation period. This increase was likely due to the rapid decomposition of materials facilitated by the PGPR consortium. Greater increases in organic matter were observed in treatments with PGPR inoculated with RP. Organic matter is crucial for healthy soil, as it consists of decomposing plant or animal material that forms the foundation for productive soil. The composition and turnover rate of organic matter impact soil structure, porosity, water infiltration, moisture retention, and nutrient availability (Khan et al., 2022b).

The application of PGPR consortia to organic-based carrier materials can significantly ($p<0.05$) improve physico-biochemical attributes, including proline (%), phenolics (71%), flavonoids (85%), and protein (94%) compared to controls. Additionally, PGPR application led to increased soil fertility and a maximum increase of 26.74% in soil organic matter (Khan, 2023). Similar findings were reported, showing that carrier-based inoculation enhanced the efficacy of multi-strain consortium inoculations and significantly ($p<0.05$) increased organic matter and other nutrients (Shaib et al., 2020).

The phosphorus (P) content in different carrier materials inoculated with the PGPR consortium and rock phosphate (RP) varied among treatments. The P content increased over the incubation period. Adding the hardly soluble P source (RP) with the bacterial consortium enhanced P content during incubation. The highest P content in the carrier materials was observed in treatments with RP throughout the incubation period. This increase in P availability was due to the bacterial strains' ability to solubilize P (Panhwar et al., 2020). These phosphate-solubilizing bacteria (PSB) were tested in *in-vitro* before inoculation in this study and demonstrated their capability to solubilize insoluble P sources. After nitrogen, phosphorus is the second most important macronutrient for plants. It is necessary for growth, development, and metabolism. Because of its complexation with metal ions, phosphorus—which is abundant in the soil in both organic and inorganic forms—is frequently inaccessible for plant uptake. Phosphate-solubilizing microbes address this issue by solubilizing insoluble phosphates through various mechanisms, such as secreting organic acids, producing enzymes, and excreting siderophores that chelate metal ions and form complexes, making phosphorus available to plants (Panhwar et al., 2020).

Insoluble phosphate can be mineralised or dissolved by a variety of soil microorganisms, releasing soluble P for plant uptake. These microorganisms can reduce the need for traditional chemical fertilisers in agriculture and are essential for increasing soil fertility. Understanding recent developments in microbial functional diversity, colonization abilities, mechanisms of action, and application methods will enhance their role as reliable components of sustainable agricultural systems (Khan et al., 2022b). Similarly, Khan et al. (2023) reported that PGPR-based carrier materials improved soil fertility by increasing mineral nutrients, particularly total nitrogen (33.10%) and available phosphorus (99.17%).

The potash content increased slightly over the incubation period across various treatments. The carrier materials used in the study contained varying levels of potash, and after the inoculation of the PGPR consortium, the potash content further increased as the incubation period progressed. Notably, higher potash levels were observed with the addition of RP when the PGPR consortium was inoculated into the carrier materials. The inoculation of the PGPR consortium led to a significant increase in organic matter and nutrients, including potassium, compared to the un-inoculated carriers. The carriers that most effectively enhanced the efficacy of the multi-strain bacterial consortia contributed to the improved quality and longer survival of the consortia, likely due to the favorable micro-environment provided by these materials for the survival and physiological activities of PGPR (Shohaib et al., 2020). Moreover, PGPR plays a critical role in crop sustainability by maintaining soil and plant health through mechanisms such as nitrogen fixation, phosphate, and potassium solubilization, among others (Khan et al., 2023).

The carrier materials inoculated with the PGPR consortium revealed the survival of PGPR bacterial strains throughout the incubation period. However, slight variations were observed among the treatments during this period. The survival of these bacterial strains is a positive indication for the materials, as bacterial survival is crucial for enhancing nutrients and organic matter through rapid decomposition. However, these inoculated carrier materials need to sustain PGPR for a longer period in various media. The survival and extended shelf life of PGPR microbes in carrier materials create an active environment, enabling quicker decomposition of the materials (Wang et al., 2016). Several materials, such as compost, biogas slurry, and zeolite, have performed well as carriers for multi-strain inoculations, effectively promoting the survival of the inoculum and improving mineral nutrient availability, which in turn enhances crop growth under various conditions.

Combined microbial strains perform better than single strains, as the synergistic combination of bacterial strains consistently leads to improved results. Additionally, using a compost-based inoculant increased total biomass production. The satisfactory survival of microbes and the enhanced plant growth observed with carrier-based inoculations may be attributed to the more suitable micro-environment provided by the carrier materials for PGPR and their activities (Espiritu et al., 2011).

Multi-strain consortia in compost and biogas slurry showed the highest results in terms of bacterial survival and attaining the greatest increases in wheat growth in a prior study. Because they are high in plant nutrients and have the physicochemical characteristics of excellent carriers, compost and other carriers offer the best conditions for inoculation microorganisms to survive (Khandare et al. 2019), this results in better plant growth and increased nutrient uptake. Applying compost and other carriers can raise the amount of organic matter surrounding the roots. As these organic materials are broken down by microorganisms, organic acids are generated, which may improve nutrient solubilisation at particular microsites. Furthermore, greater populations of bacteria in the soil rhizosphere or other substrates can be supported by organic carriers acting as substrates (Ma, 2019).

CONCLUSION

Overall, it can be concluded that the selected four potential PGPR strains, two in each of were combined in PGPR consortia (conso-I and conso-II). Both bacterial consortia demonstrated the ability to survive and solubilize phosphorus in liquid media during incubation, however, conso-I performed better than conso-II. The application of multi-strain bacterial inoculants to various solid carrier materials enhanced the quality of the carriers by increasing organic matter content, slightly improving mineral nutrient levels, and lowering the pH. Inoculating PGPR consortia into different carrier materials resulted in an increase in available phosphorus from the less soluble rock phosphate during the incubation period. The performance of different carrier materials for bio-inoculants was statistically similar. The survival of PGPR strains in various carrier materials indicates that these materials are suitable for bacterial survival and can improve carrier quality through the rapid decomposition of carriers. The effectiveness of the multi-strain consortium in natural soil environments will further confirm these results.

ACKNOWLEGEMENT

We gratefully acknowledge the Nuclear Institute of Agriculture (NIA), Pakistan Atomic Energy Commission, and Sindh Agriculture University, Tandojam, for providing technical support, laboratory facilities, and assistance in conducting the experiments.

Declaration of Funding

This research did not receive any specific funding.

Data Availability

The datasets analyzed during this study are included in this manuscript.

Ethics, Consent to Participate, and Consent to Publish declarations

Not applicable

REFERENCES

Afzal, I. M. R. A. N., Shinwari, Z. K., & Iqrar, I. (2015). Selective isolation and characterization of agriculturally beneficial endophytic bacteria from wild hemp using canola. *Pakistan Journal of Botany., 47*(5), 1999-2008.

Ali, A., Ali, A., Akhtar, J., & Yaseen, M. (2010). Effects of phosphorus in combination with rhizobium inoculation on growth and yield parameters of mungbean. *Crop & Environment., 1*, 53-56.

Alori, E. T., Glick, B. R., & Babalola, O. O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in microbiology*, *8*, 971.

Alotaibi, F., St-Arnaud, M., & Hijri, M. (2022). In-depth characterization of plant growth promotion potentials of selected alkanes-degrading plant growth-promoting bacterial isolates. *Frontiers in Microbiology*, *13*, 863702.

Amanullah, Khan, I., Jan, A., Jan, M. T., Khalil, S. K., Shah, Z., & Afzal, M. (2015). Compost and nitrogen management influence productivity of spring maize (*Zea mays L.)* under deep and conventional tillage systems in Semi-arid regions. *Communications in Soil Science and Plant Analysis*, *46*(12), 1566^{-1}578.

Amri, M., Rjeibi, M. R., Gatrouni, M., Mateus, D. M., Asses, N., Pinho, H. J., & Abbes, C. (2023). Isolation, identification, and characterization of phosphate-solubilizing bacteria from Tunisian soils. *Microorganisms*, *11*(3), 783.

Antoun, H. (2012). Beneficial microorganisms for the sustainable use of phosphates in agriculture. *Procedia Engineering*, *46*, 62-67.

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., ... & Smith, D. L. (2018). Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in Plant Science*, *9*, 402666.

Balducci, E., Papi, F., Capialbi, D. E., & Del Bino, L. (2023). Polysaccharides' structures and functions in biofilm architecture of antimicrobial-resistant (AMR) pathogens. *International Journal of Molecular Sciences*, *24*(4), 4030.

Baliah, T. N., & Priya, A. S. (2017). Effect of bioformulations of Phosphate Solubilizing Bacteria (PSB) on the Growth and Biochemical Characters of the (*Gossypium hirsutum)* and (*Zea mays)*. *International Journal of Environment, Agriculture and Biotechnology (IJEAB)*, *2*(6), 3229-3236.

Barrow, N. J. (2015). Soil phosphate chemistry and the P-sparing effect of previous phosphate applications. *Plant and Soil*, *397*(1), 401-409.

Bhattacharya, A., & Bhattacharya, A. (2021). Mineral nutrition of plants under soil water deficit condition: A Review. *Soil Water Deficit and Physiological Issues in Plants*, 287-391.

Calvo, P., Nelson, L., & Kloepper, J. W. (2014). Agricultural uses of plant biostimulants. *Plant and Soil*, *383*, 3-41.

Carvalhais, L. C., Dennis, P. G., Badri, D. V., Kidd, B. N., Vivanco, J. M., & Schenk, P. M. (2015). Linking jasmonic acid signaling, root exudates, and rhizosphere microbiomes. *Molecular Plant-Microbe Interactions*, *28*(9), 1049-1058.

Cavite, H. J. M., Mactal, A. G., Evangelista, E. V., & Cruz, J. A. (2021). Biochemical characteristics and inoculation effects of multi-trait plant growth-promoting rhizobacteria on upland rice (*Oryza sativa* L. cv PSB Rc23) seedling growth. *Archives of Microbiology*, *203*(6), 3533-3540.

Chandini, B., Randeep, K., Ravendra, K., & Pantnagar, O. P. (2019). The Impact of Chemical Fertilizers on our Environment and Ecosystem. P 69-86. *Research Trends in Environmental Sciences, 2nd Edition, AkiNik Publications, New Delhi, India*.

Chandler, D., Davidson, G., Grant, W. P., Greaves, J., & Tatchell, G. M. (2008). Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory sustainability. *Trends in Food Science & Technology*, *19*(5), 275-283.

Cheng, Y., Narayanan, M., Shi, X., Chen, X., Li, Z., & Ma, Y. (2023). Phosphate-solubilizing bacteria: Their agroecological function and optimistic application for enhancing agro-productivity. *Science of The Total Environment*, 166468.

Cordell, D. E. N. S. I. T. Y., & Neset, T. S. (2014). Phosphorus vulnerability: a qualitative framework for assessing the vulnerability of national and regional food systems to the multi-dimensional stressors of phosphorus scarcity. *Global Environmental Change*, *24*, 108-122.

Doydora, S., Gatiboni, L., Grieger, K., Hesterberg, D., Jones, J. L., McLamore, E. S., ... & Duckworth, O. W. (2020). Accessing legacy phosphorus in soils. *Soil Systems*, *4*(4), 74.

Elhaissoufi, W., Ghoulam, C., Barakat, A., Zeroual, Y., & Bargaz, A. (2022). Phosphate bacterial solubilization: a key rhizosphere driving force enabling higher P use efficiency and crop productivity. *Journal of Advanced Research*, *38*, 13-28.

Espiritu, B. M. (2011). Use of compost with microbial inoculation in container media for mungbean (*Vigna radiata L. Wilckzek)* and pechay (*Brassica napus L.*). *Journal of the International Society for Southeast Asian Agricultural Sciences, 17*(1), 160-168.

Fasusi, O. A., Cruz, C., & Babalola, O. O. (2021). Agricultural sustainability: microbial biofertilizers in rhizosphere management. *Agriculture*, *11*(2), 163.

Fink, J. R., Inda, A. V., Tiecher, T., Barrón, V. (2016). Iron oxides and organic matter on soil phosphorus availability. *Ciencia e agrotecnologia*, *40*, 369-379.

Gerard, F., 2016. Clay minerals, iron/aluminum oxides, and their contribution to phosphate sorption in soils – a myth revisited. *Geoderma. 262,* 213–226.

Giauque, H., Connor, E. W., & Hawkes, C. V. (2019). Endophyte traits relevant to stress tolerance, resource use and habitat of origin predict effects on host plants. *New Phytologist*, *221*(4), 2239-2249.

Glick, B. R., Patten, C. L., Holguin, G., & Penrose, D. M. (1999). *Biochemical and genetic mechanisms used by plant growth promoting bacteria*.

Government of Pakistan, (2015). Economics survey of Pakistan $2014^{-1}5$, Ministry of Food, Agriculture and Livestock (Federal Bureau of Statistics), Islamabad (P.23-44).

Gulnaz, Y., Fathima, P. S., Denesh, G. R., Kulmitra, A. K., & Shivrajkumar, H. S. (2017). Effect of plant growth promoting rhizobacteria (PGPR) and PSB on root parameters, nutrient uptake and nutrient use efficiency of irrigated maize under varying levels of phosphorus. *Journal of Entomology Zoology Studies*, *5*(6), 166^{-1}69.

Gupta, A., Bano, A., Rai, S., Sharma, S., Pathak, N. (2022). Selection of carrier materials to formulate bioinoculant package for promoting seed germination. *Letters in Applied NanoBioScience*, *12*, 65.

Hasan, A., Tabassum, B., Hashim, M., & Khan, N. (2024). Role of plant growth promoting rhizobacteria (PGPR) as a plant growth enhancer for sustainable agriculture: A review. *Bacteria*, *3*(2), 59-75.

Hassanisaadi, M., Shahidi Bonjar, G. H., Hosseinipour, A., Abdolshahi, R., Ait Barka, E., & Saadoun, I. (2021). Biological control of Pythium aphanidermatum, the causal agent of tomato root rot by two Streptomyces root symbionts. *Agronomy*, *11*(5), 846.

Hawkesford, M. J., Cakmak, I., Coskun, D., De Kok, L. J., Lambers, H., Schjoerring, J. K., & White, P. J. (2023). Functions of macronutrients. In *Marschner's Mineral Nutrition of Plants* (pp. 201-281). Academic press.

Hu, B., Jiang, Z., Wang, W., Qiu, Y., Zhang, Z., Liu, Y., ... & Chu, C. (2019). Nitrate–NRT1. 1B–SPX4 cascade integrates nitrogen and phosphorus signalling networks in plants. *Nature Plants*, *5*(4), 401-413.

Hussain, A., A. Ali, J. Akhter and M. Yasin. 2010. Effect of phosphorous in combination with Rhizobium inoculation on growth and yield parameters of Mungbean (*Vignar adiata* L.) *Crop & Environment.,* 1: 53-56.

Hussain, A., Iqbal, Z., Aurangzaib, M., Naeem, M., Mustafa, S., Shoaib, M., & Khalid, M. (2022). Comparative effectiveness of plant growth promoting rhizobacteria and various organic carriers on wheat growth, physiology, antioxidative activities and rhizosphere properties. *Pakistan Journal of Botany*, *54*(1), 317-324.

Hussain, A., Iqbal, Z., Aurangzaib, M., Naeem, M., Mustafa, S., Shoaib, M., & Khalid, M. (2022). Comparative effectiveness of plant growth promoting rhizobacteria and various organic carriers on wheat growth, physiology, antioxidative activities and rhizosphere properties. *Pakistan Journal of Botany*, *54*(1), 317-324.

Ibrahim, M., Iqbal, M., Tang, Y. T., Khan, S., Guan, D. X., & Li, G. (2022). Phosphorus mobilization in plant–soil environments and inspired strategies for managing phosphorus: A review. *Agronomy*, *12*(10), 2539.

Ikhajiagbe, B., Anoliefo, G. O., Olise, O. F., Rackelmann, F., Sommer, M., & Adekunle, I. J. (2020). Major phosphorus in soils is unavailable, yet critical for plant development. *Notulae Scientia Biologicae*, *12*(3), 500-535.

Johan, P. D., Ahmed, O. H., Omar, L., & Hasbullah, N. A. (2021). Phosphorus transformation in soils following co-application of charcoal and wood ash. *Agronomy*, *11*(10), 2010.

Kalayu, G. (2019). Phosphate solubilizing microorganisms: promising approach as biofertilizers. *International Journal of Agronomy*, *2019*(1), 4917256.

Kavitha, P., & Srinivasan, S. (2013). Ranjini. Individual and combined effect of biofertilizer, chemical fertilizer and vermicompost on Amaranthus tristis. *International Journal of Pharmaceutical Review and Research*, *20*(2), 190-195

Kaya, C., Şenbayram, M., Akram, N. A., Ashraf, M., Alyemeni, M. N., & Ahmad, P. (2020). Sulfur-enriched leonardite and humic acid soil amendments enhance tolerance to drought and phosphorus deficiency stress in maize (*Zea mays L.*). *Scientific Reports*, *10*(1), 6432.

Khan, A., Khan, R. A., Ali, M., & Shinwari, Z. K. (2023). Impact of plant growth promoting rhizobacteria biofertilzers on biochemical attributes, antioxidant activities, nutritional values and productivity of maize. *Pakistan. Journal of Botany, 55(6),* 2057-2066.

Khan, M. S., Rizvi, A., Ahmed, B., & Lee, J. (2022). Phosphate biofertilizers: Recent trends and new perspectives. *Trends of Applied Microbiology for Sustainable Economy*, 421-461.

Khan, M. Y., Nadeem, S. M., Sohaib, M., Waqas, M. R., Alotaibi, F., Ali, L., Zahir ZA, & Al-Barakah, F. N. (2022). Potential of plant growth promoting bacterial consortium for improving the growth and yield of wheat under saline conditions. *Frontiers in Microbiology*, *13*, 958522.

Khan, M. Y., Zahir, Z. A., Asghar, H. N., & Waraich, E. A. (2017). Preliminary investigations on selection of synergistic halotolerant plant growth promoting rhizobacteria for inducing salinity tolerance in wheat. *Pakistan Journal of Botany*, *49*(4), $1541^{-1}551$.

Khandare, R. N., Chandra, R., Pareek, N., & Raverkar, K. P. (2020). Carrier-based and liquid bioinoculants of Azotobacter and PSB saved chemical fertilizers in wheat (*Triticum aestivum* L.) and enhanced soil biological properties in Mollisols. *Journal of Plant Nutrition*, *43*(1), 36-50.

Kim, K. Y., Jordan, D., & McDonald, G. A. (1997). Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. *Biology and Fertility of Soils*, *26*, 79-87.

Kloepper, J. W., Lifshitz, R., & Zablotowicz, R. M. (1989). Free-living bacterial inocula for enhancing crop productivity. *Trends in Biotechnology*, *7*(2), 39-44.

Kumar, A., Patel, J. S., & Meena, V. S. (2018). Rhizospheric microbes for sustainable agriculture: an overview. *Role of Rhizospheric Microbes in Soil: Volume 1: Stress Management and Agricultural Sustainability*, 1-31.

Kumar, R., & Chandra, R. (2008). Influence of PGPR and PSB on Rhizobium leguminosarum bv. viciae strain competition and symbiotic performance in lentil. *World Journal of Agricultural Sciences*, *4*(3), 297-301.

Kumar, S., Sindhu, S. S., & Kumar, R. (2022). Biofertilizers: An ecofriendly technology for nutrient recycling and environmental sustainability. *Current Research in Microbial Sciences*, *3*, 100094.

Ladha, J. K., Peoples, M. B., Reddy, P. M., Biswas, J. C., Bennett, A., Jat, M. L., & Krupnik, T. J. (2022). Biological nitrogen fixation and prospects for ecological intensification in cereal-based cropping systems. *Field Crops Research*, *283*, 108541.

Laghari, F. R., Panhwar, Q. A., Jamro, G. M., Ali, A., Rajpar, I., & Depar, N. (2024). Assessment of appropriate carrier material for the growth and survival of phosphate-solubilizing bacteria (*Enterobacter cloacae*) and their potential for improving growth of chilli (*Capsicum annuum* L.). *Pakistan Journal of Agricultural Sciences*, *61*(1).

Liu, S., Meng, J., Jiang, L., Yang, X., Lan, Y., Cheng, X., & Chen, W. (2017). Rice husk biochar impacts soil phosphorous availability, phosphatase activities and bacterial community characteristics in three different soil types. *Applied Soil Ecology*, *116*, 12-22.

Lugtenberg, B., & Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology*, *63*(1), 541-556.

Ma, Y. (2019). Seed coating with beneficial microorganisms for precision agriculture. *Biotechnology Advances*, *37*(7), 107423.

Malhotra, H., Vandana, Sharma, S., & Pandey, R. (2018). Phosphorus nutrition: plant growth in response to deficiency and excess. *Plant Nutrients and Abiotic Stress Tolerance*, 171-190.

Mantelin, S., Touraine, B. (2004). Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. *Journal of Experimental Botany*, *55*(394), 27-34.

Mohammadi, K., Sohrabi, Y. (2012). Bacterial biofertilizers for sustainable crop production: a review. *ARPN Journal of Agriculture and Biological Science*, *7*(5), 307-316.

Mondal, S., Dutta, S., Banerjee, A., Banerjee, S., Datta, R., Roy, P., Podder, A., Dasgupta, P., & Saha, D. (2017). Production and application of phosphate solubilizing bacteria as biofertilizer: field trial at maize field, Uchalan, Burdwan District, West Bengal. *International Journal of Environmental and Agriculture Research* 3:1-9.

Mukhtar, S., Shahid, I., Mehnaz, S., & Malik, K. A. (2017). Assessment of two carrier materials for phosphate solubilizing biofertilizers and their effect on growth of wheat (*Triticum aestivum* L.). *Microbiological Research*, *205*, 107-117.

Naeem, A., Akhtar, M., & Ahmad, W. (2013). Optimizing available phosphorus in calcareous soils fertilized with diammonium phosphate and phosphoric acid using Freundlich adsorption isotherm. *The Scientific World Journal*, *2013*(1), 680257.

Naher, U. A., Othman, R., Latif, M. A., Panhwar, Q. A., Amaddin, P. A. M., & Shamsuddin, Z. H. (2013). Biomolecular characterization of diazotrophs isolated from the tropical soil in Malaysia. *International journal of molecular sciences*, *14*(9), 17812-17829.

Narractives. (2023). *The Future of the Fertilizer Sector in Pakistan.* https://narratives.com.pk/featured/the-futureof-the-fertilizer-sector-in-pakistan.

Nierves, M. C. P., & Salas, F. M. (2015). Assessment of soil phosphorus and phosphorus fixing capacity of three vegetable farms at Cabintan, Ormoc city, Leyte. *World Journal of Agricultural Research*, *3*(2), 70-73.

Olanrewaju, O. S., Glick, B. R., & Babalola, O. O. (2017). Mechanisms of action of plant growth promoting bacteria. *World Journal of Microbiology and Biotechnology*, *33*, 1-16.

Oldroyd, G. E., & Leyser, O. (2020). A plant's diet, surviving in a variable nutrient environment. *Science*, *368*(6486), eaba0196.

Packialakshmi, N., & Aliya, R. T. (2014). Comparative study of vermicast and charcoal used as carrier inoculums to the biofertilizer preparation. *BMR Biotechnology*, *1*(1), 1-6.

Panhwar, Q. A., Naher, U. A., Jusop, S., Othman, R., Latif, M. A., & Ismail, M. R. (2014). Biochemical and molecular characterization of potential phosphate-solubilizing bacteria in acid sulfate soils and their beneficial effects on rice growth. *PlOS ONE*, *9*(10), e97241.

Panhwar, Q. A., Naher, U. A., Shamshuddin, J., & Ismail, M. R. (2020). Effects of biochar and ground magnesium limestone application, with or without bio-fertilizer addition, on biochemical properties of an acid sulfate soil and rice yield. *Agronomy*, *10*(8), 1100.

Panhwar, Q. A., Radziah, O., Sariah, M., & Ismail, M. R. (2009). Solubilization of different phosphate forms by phosphate solubilizing bacteria isolated from aerobic rice. *International Journal of Agriculture and Biology*, *11*(6), 667-673.

Pankievicz, V. C., Irving, T. B., Maia, L. G., & Ané, J. M. (2019). Are we there yet? The long walk towards the development of efficient symbiotic associations between nitrogen-fixing bacteria and non-leguminous crops. *BMC Biology*, *17*(1), 99.

Perveen, R., Hussain, A., Ditta, A., Dar, A., Aimen, A., Ahmad, M., Alataway, A., Dewider, Z. A., & Mattar, M. A. (2022). Growth and yield of Okra exposed to a consortium of rhizobacteria with different organic carriers under controlled and natural field conditions. *Horticulturae*, *9*(1), 8.

Pradhan, N., & Sukla, L. B. (2006). Solubilization of inorganic phosphates by fungi isolated from agriculture soil. *African Journal of Biotechnology*, *5*(10).

Prathap, V., Kumar, A., Maheshwari, C., & Tyagi, A. (2022). Phosphorus homeostasis: Acquisition, sensing, and long-distance signaling in plants. *Molecular Biology Reports*, *49*(8), 8071-8086.

Radzki, W., Gutierrez Mañero, F. J., Algar, E., Lucas García, J. A., García-Villaraco, A., & Ramos Solano, B. (2013). Bacterial siderophores efficiently provide iron to iron-starved tomato plants in hydroponics culture. *Antonie Van Leeuwenhoek*, *104*, 321-330.

Rajput, L., Imran, A., Mubeen, F., & Hafeez, F. Y. (2018). Wheat (*Triticum aestivum L.*) growth promotion by halo-tolerant PGPR-consortium. *Soil Environment*, *37*(2), 178-189.

Rehan, M., Al-Turki, A., Abdelmageed, A. H., Abdelhameid, N. M., & Omar, A. F. (2023). Performance of Plant-Growth-Promoting Rhizobacteria (PGPR) Isolated from Sandy Soil on Growth of Tomato (*Solanum lycopersicum* L.). *Plants*, *12*(8), 1588.

Safdar, H., Jamil, M., Hussain, A., Albalawi, B. F. A., Ditta, A., Dar, A., Aimen, A., Ahmed, T. H., Nazir, Q., & Ahmad, M. (2022). The effect of different carrier materials on the growth and yield of spinach under pot and field experimental conditions. *Sustainability*, *14*(19), 12255.

Sakin, E. (2024). The Advantages and Disadvantages of Calcareous Soils.

Santoro, V., Schiavon, M., & Celi, L. (2024). Role of soil abiotic processes on phosphorus availability and plant responses with a focus on strigolactones in tomato plants. *Plant and Soil*, *494*(1), 1-49.

Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M., Glick, B. R. (2016). Plant growth-promoting bacterial endophytes. *Microbiological Research*, *183*, 92-99.

Satyaprakash, M., Nikitha, T., Reddi, E. U. B., Sadhana, B., Vani, S. S. (2017). Phosphorous and phosphate solubilizing bacteria and their role in plant nutrition. *International Journal of Current Microbiology and Applied Sciences*, *6*(4), 2133-2144.

Schlievert, P.M. and Peterson, M.L., 2012. Glycerol monolaurate antibacterial activity in broth and biofilm cultures. *PloS one,* 7(7), p.e40350.

Serra, B., Zhang, J., Morales, M. D., de Prada, A. G. V., Reviejo, A. J., & Pingarron, J. M. (2008). A rapid method for detection of catalase-positive and catalase-negative bacteria based on monitoring of hydrogen peroxide evolution at a composite peroxidase biosensor. *Talanta*, *75*(4), 1134-1139.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., & Gobi, T. A. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springer Plus*, *2*, 1-14.

Siddiq, S., Saleem, U., Ahmad, K., Anayat, A., Affan, Q. M., Anwar, M. F., Nazir, H., & Asghar, N. (2018). Comparison of conventional and non-conventional carriers for bacterial survival and plant growth. *International Journal Agriculture Innovations Research, 6*(4), 126-129.

Sivasakthivelan, P., & Saranraj, P. (2013). *Azospirillum* and its formulations: a review. *International Journal of Microbiological Research*, *4*(3), 275-287.

Sohaib, M., Zahir, Z. A., Khan, M. Y., Ans, M., Asghar, H. N., Yasin, S., & Al-Barakah, F. N. (2020). Comparative evaluation of different carrier-based multi-strain bacterial formulations to mitigate the salt stress in wheat. *Saudi Journal of Biological Sciences*, *27*(3), 777-787.

Sørensen, J., & Sessitsch, A. (2007). Plant-associated bacteria lifestyle and molecular interactions. *Modern Soil Microbiology*, *2*, 211-236.

Tahir, M., Shahid, M., Nawaz, F., Ahmad, I., Ijaz, M., Farooq, A. B. U, & Abbas, Y. (2022). Efficacy of organic-based carrier material for plant beneficial rhizobacteria application in okra under normal and salt-affected soil conditions. *Journal of Applied Microbiology*, *133*(2), 943-959.

Tahir, M., Shahid, M., Nawaz, F., Ahmad, I., Ijaz, M., Farooq, A. B. U., Akram, M., Khalid, U., Naqqash, T., Mehmood, S., Mubeen, M., Sarfaraz, M., & Abbas, Y. (2022). Efficacy of organic-based carrier material for plant beneficial rhizobacteria application in okra under normal and salt-affected soil conditions. *Journal of Applied Microbiology*, *133*(2), 943-959.

Timofeeva, A., Galyamova, M., & Sedykh, S. (2022). Prospects for using phosphate-solubilizing microorganisms as natural fertilizers in agriculture. *Plants*, *11*(16), 2119.

Upadhyay, S. K., Singh, J. S., Saxena, A. K., & Singh, D. P. (2012). Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions. *Plant Biology*, *14*(4), 605-611.

van de Wiel, C. C., van der Linden, C. G., & Scholten, O. E. (2016). Improving phosphorus use efficiency in agriculture: opportunities for breeding. *Euphytica*, *207*, 1-22.

Vance, C. P. (2011). Phosphorus as a critical macronutrient. *The Molecular and Physiological Basis of Nutrient Use Efficiency in Crops*, 227-264.

Wang, C., Dong, D., Wang, H., Müller, K., Qin, Y., Wang, H., & Wu, W. (2016). Metagenomic analysis of microbial consortia enriched from compost: new insights into the role of Actinobacteria in lignocellulose decomposition. *Biotechnology for Biofuels*, *9*, 1-17.

Wang, R., Guo, S., Li, N., Li, R., Zhang, Y., Jiang, J., & Zhao, M. (2015). Phosphorus accumulation and sorption in calcareous soil under long-term fertilization. *Plos One*, *10*(8), e0135160.

Youssef, M. M. A., & Eissa, M. F. M. (2014). Biofertilizers and their role in management of plant parasitic nematodes. A review. *Journal of Biotechnology and Pharmaceutical Research*, *5*(1), 1-6.

Zafar-ul-Hye, M., Bhutta, T. S., Shaaban, M., Hussain, S., Qayyum, M. F., Aslam, U., & Zahir, Z. A. (2019). Influence of plant growth promoting rhizobacterial inoculation on wheat productivity under soil salinity stress. *Phyton*, *88*(2), 119.

Zhang, B. X., Li, P. S., Wang, Y. Y., Wang, J. J., Liu, X. L., Wang, X. Y., & Hu, X. M. (2021). Characterization and synthesis of indole-3-acetic acid in plant growth promoting *(Enterobacter* sp*.). RSC advances*, *11*(50), 31601-31607.

List of Tables:

Table 1. EC and pH in NBRIP as P- source inoculated with PGPR Consortia

Incubation period (hrs)	EC (dSm^{-1})			pH		
	Control	Conso-I	Conso-II	Control	Conso-I	Conso-II
0	2.1c	3.3c	3.7a	7.0d	7.0a	7.0a
24	2.3b	3.6b	3.6a	7.1c	5.9b	5.4b
48	2.4a	3.7a	3.3c	7.3a	5.5c	4.9c
72	2.4a	3.8a	3.5b	7.2b	5.1d	4.8d
96	2.3b	3.6b	3.6a	7.3a	4.9e	4.7e

*Conso=consortium, EC=electricity conductivity
Mean within the same column followed by the same letter are significantly different at P<0.005.

Table 2. Electrical conductivity (EC) and pH in RP as P- source inoculated with PGPR Consortia

Incubation period (hrs)	EC (dSm^{-1})			pH		
	Control	Conso-I	Conso-II	Control	Conso-I	Conso-II
Control	2.1c	3.02d	3.15d	7.0d	7.0a	7.0a
24	2.2b	3.45a	3.23cd	7.1c	5.4b	5.3b
48	2.3a	3.41a	3.32a	7.2b	4.9c	4.8c
72	2.2b	3.35b	3.28b	7.2b	4.7c	4.6c
96	2.2b	3.23c	3.24c	7.3a	4.2d	4.1d

*Conso=consortium
Mean within the same column followed by the same letter are significantly different at P<0.005.

Table 3. P-Solubilization efficiency (%) in NBRIP and RP media inoculated with Conso-I & II.

Incubation period (hrs)	P-Solubilization efficiency (%)			
	NBRIP		RP	
	Conso-I	Conso-II	Conso-I	Conso-II
24	21.25d	23.33c	4.95c	4.69b
48	33.33c	35.43b	5.15b	4.75b
72	36.36b	37.03b	5.28a	4.81a
96	46.28a	41.52a	5.29a	4.82a

Means with in the same column followed by the same letters are not significantly different at P<0.05

Table 4. Changes of pH in various carrier materials and RP inoculated with PGPR consortium during the incubation period of 90 days

Treatments	pH		
	After 30 days of inoculation	After 60 days of inoculation	After 90 days of inoculation
CM1+RP0	8.0c	7.7c	7.7ab
CM2+RP0	8.2ab	7.7c	7.6b
CM3+RP0	8.3a	7.8b	7.8a
CM1+RP1	8.3a	7.7c	7.6b
CM2+RP1	8.2ab	7.8b	7.7ab
CM3+RP1	7.9c	7.7c	7.3d
CM1+RP2	8.1b	7.8b	7.4c
CM2+RP2	8.3a	7.8b	7.6b
CM3+RP2	8.2ab	7.7c	7.3d
CM1+RP3	8.3a	7.9a	7.4c
CM2+RP3	8.2ab	7.7c	7.5c
CM3+RP3	8.1b	7.9a	7.4c

*CM=carrier material, RP=rock phosphate

Mean within the same column followed by the same letter are significantly different at P<0.005.

Table 5. Changes of organic matter contents in various carrier materials inoculated with PGPR consortium during the incubation period

Treatments	Organic matter contents (%)		
	After 30 days	After 60 days	After 90 days
CM1+RP0	38.23d	41.87e	46.34e
CM2+RP0	39.51d	45.18c	42.51f
CM3+RP0	41.72c	47.42a	48.43c
CM1+RP1	36.34d	43.82d	48.62c
CM2+RP1	33.06e	40.32e	49.24b
CM3+RP1	45.62a	45.22c	50.43a
CM1+RP2	42.02c	41.35e	48.28c
CM2+RP2	45.73a	46.32b	49.35b
CM3+RP2	44.56b	47.72a	50.48a
CM1+RP3	45.05a	45.23c	47.01d
CM2+RP3	42.23c	47.23a	50.22a
CM3+RP3	45.22a	47.34a	50.01a

*CM=carrier material, RP=rock phosphate

Mean within the same column followed by the same letter are significantly different at P<0.005.

Table 6. Changes of phosphate contents in various carrier materials (CM)

Treatments	Available phosphate (%)		
	After 30 days	After 60 days	After 90 days
CM1+RP0	0.57f	0.63h	0.69h
CM2+RP0	0.42g	0.55i	0.63i
CM3+RP0	0.58f	0.67h	0.72h
CM1+RP1	2.47e	3.69f	4.46g
CM2+RP1	2.43e	3.93e	4.37g

CM3+RP1	2.85d	3.40g	4.65f
CM1+RP2	2.57e	3.06h	6.99d
CM2+RP2	2.47e	4.82bc	5.97e
CM3+RP2	3.01c	4.37d	7.64c
CM1+RP3	3.52b	4.94b	8.19b
CM2+RP3	2.99c	4.64c	7.73c
CM3+RP3	3.40a	5.34a	8.73a

840 *CM=carrier material, RP=rock phosphate

841 Mean within the same column followed by the same letter are significantly different at P<0.005.

842 **Table 7. Changes of potassium contents in various carrier materials**

Treatments	Potash (%)		
	After 30 days	After 60 days	After 90 days
CM1+RP0	7.60e	7.82e	9.22e
CM2+RP0	6.40f	7.21f	8.34f
CM3+RP0	7.81e	7.65e	9.06e
CM1+RP1	8.00d	8.44d	9.63d
CM2+RP1	8.40c	8.62d	9.74d
CM3+RP1	8.66b	9.23c	10.60c
CM1+RP2	8.69ab	9.64a	11.02b
CM2+RP2	8.06d	9.45b	10.65c
CM3+RP2	8.25d	9.61a	11.32ab
CM1+RP3	8.24d	9.64a	10.67c
CM2+RP3	8.87a	9.52ab	10.44c
CM3+RP3	8.83a	9.73a	11.43a

843 *CM=carrier material, RP=rock phosphate

844 Mean within the same column followed by the same letter are significantly different at P<0.005.

845

846

List of Figures:

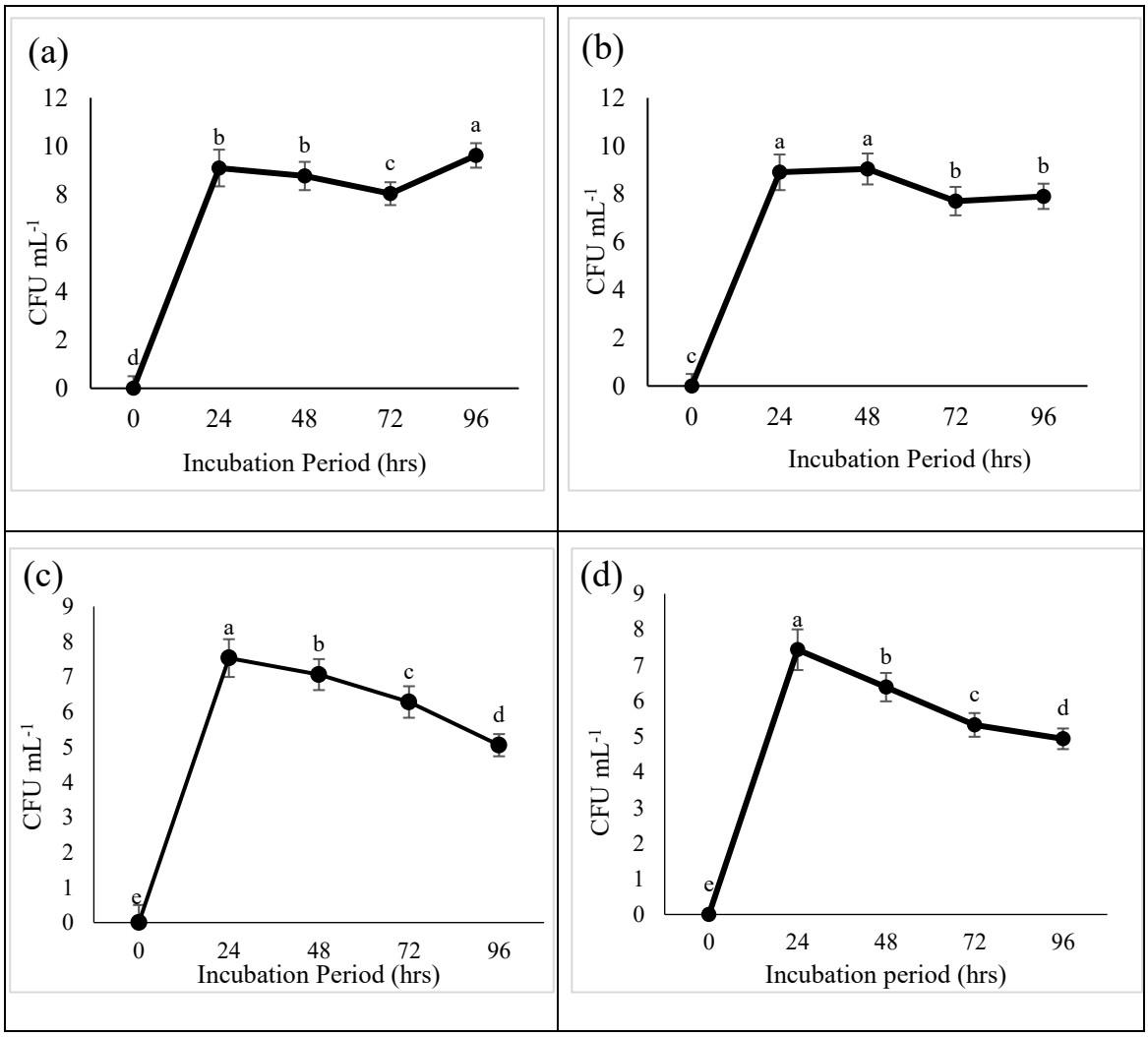
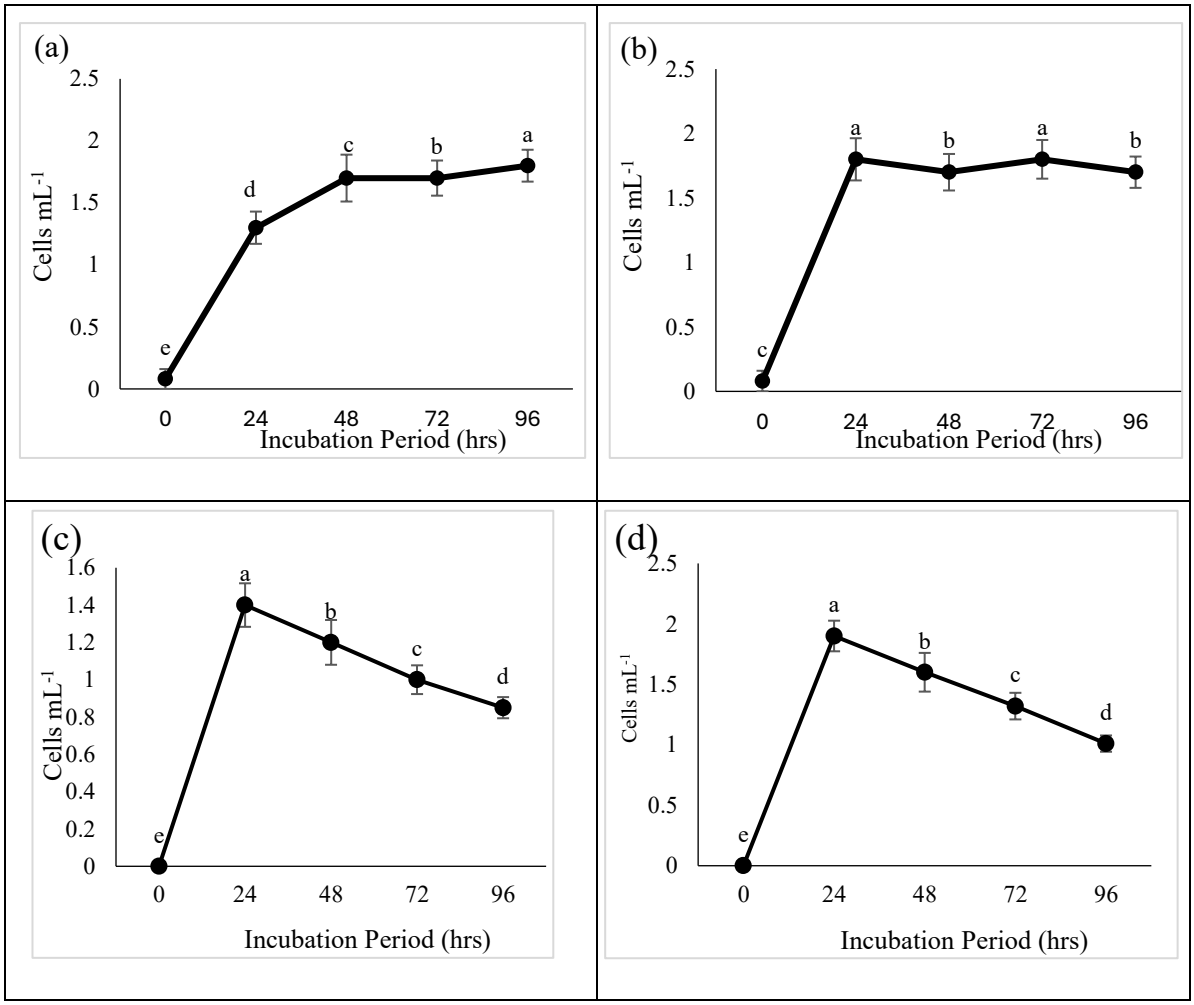


Figure 1. Determination of total bacterial count (a) Conso-I in NBRIP (b) Conso-II in NBRIP and (c) Conso-I in RP (d) Conso-II in RP

Means with in the same column followed by the same letters are not significantly different at P<0.05.

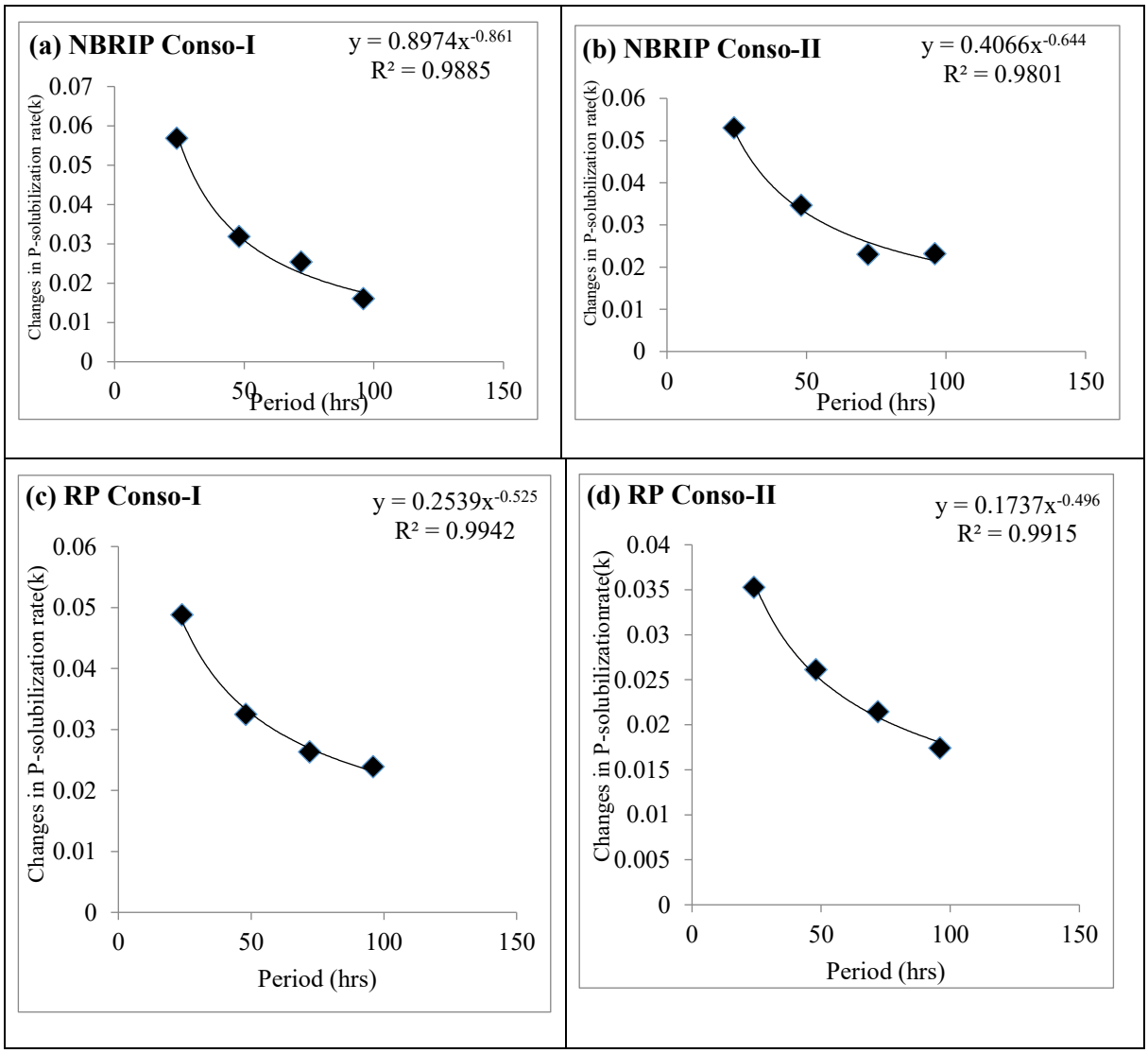


853 **Figure 2. Optical Density of NBRIP incubated (a) Conso-I (b) Conso II and RP**
854 **inoculated (c) Conso-I (d) Conso II**

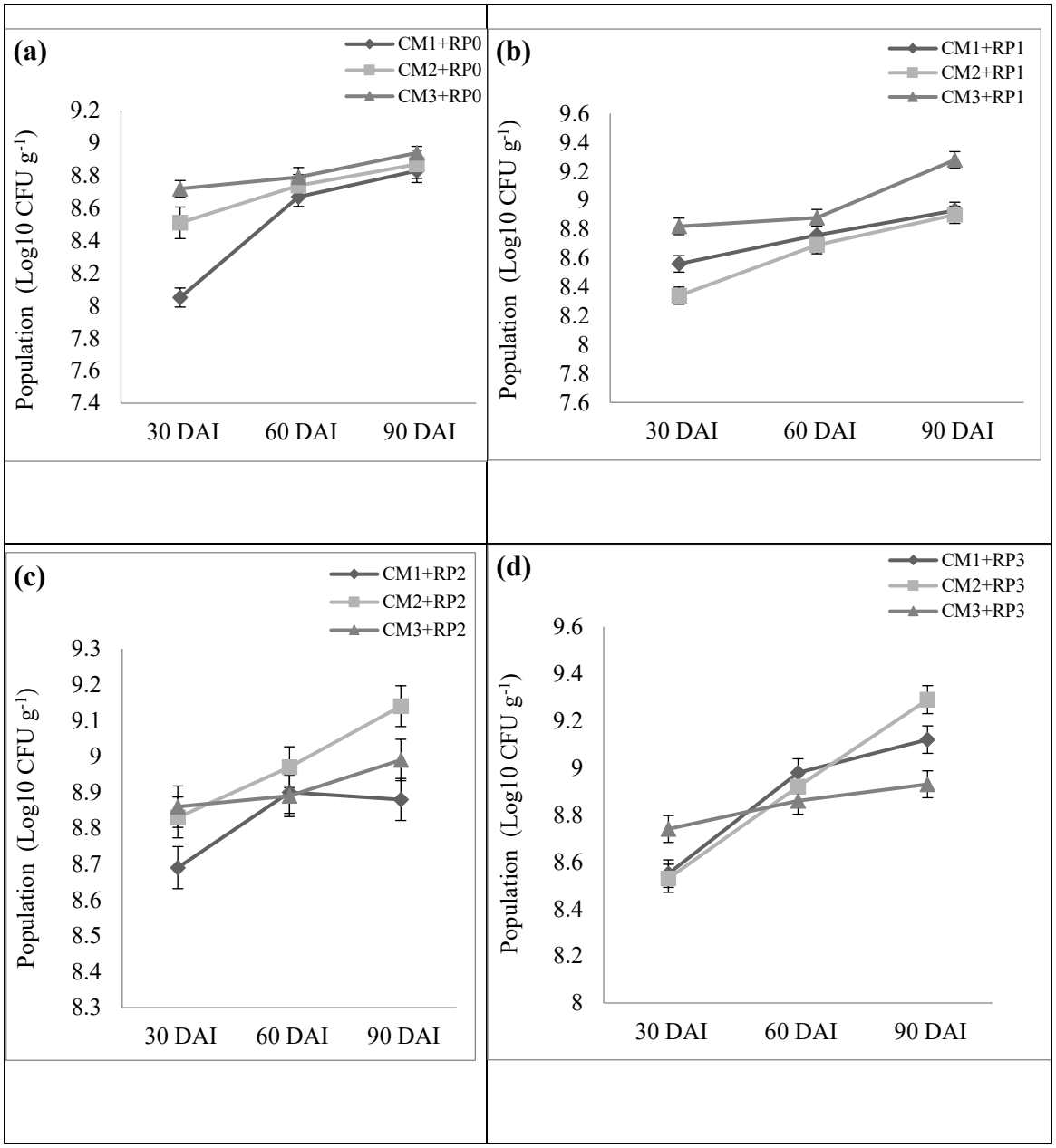
855 Means with in the same column followed by the same letters are not significantly different at P<0.05.

856

857



858 **Figure 3. Kinetics of P solubilization in various broths inoculated with Conso-I and**
859 **II.**
860



861 **Figure 4. Changes of total bacterial count in various carrier materials**

862 Where: CM=carrier material, RP=rock phosphate, DAI= days after inoculation

863

864