Impact of Wheat-Derived Rhizobacteria on the Maize Development from District Bajaur, Khyber Pakhtunkhwa, Pakistan

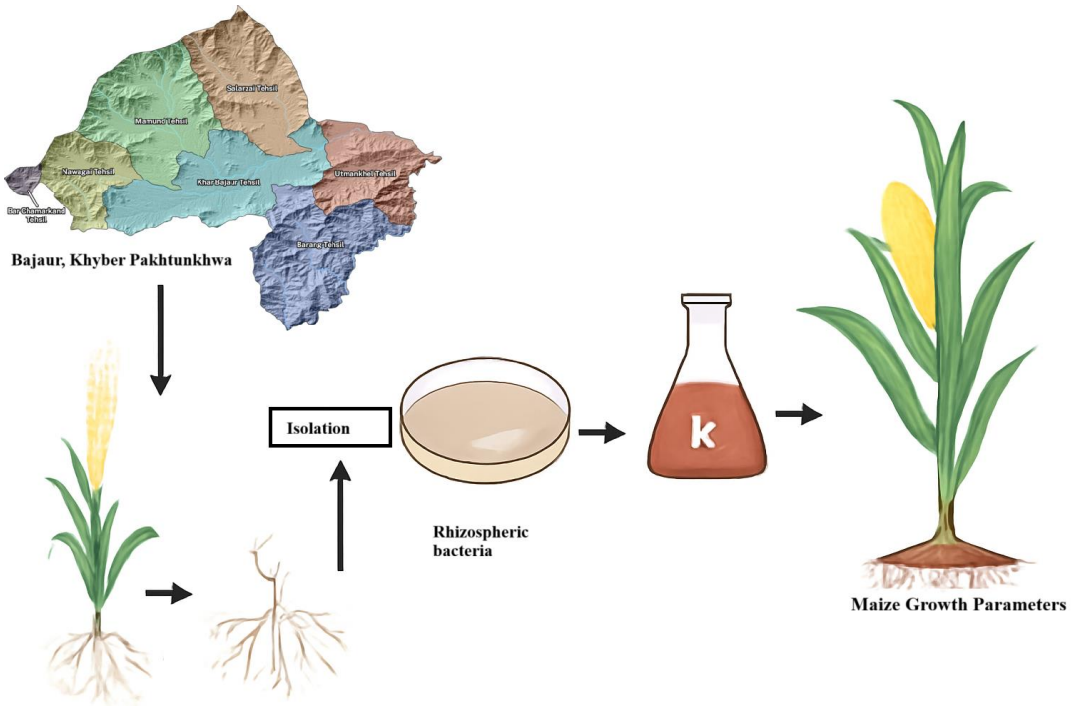
Asia Bibi[1,], Saima Ashraf[2], Nosheen Azhar[3], Sidra Qayyum[4], Masab Saeed[5], Rida Zameer[6], Hifsa Arif[7], Syed Azhar Shah Kazmi[8], Asim Shahzad[9],*

1. *[*]Department of Botany, Women University of Azad Jammu & Kashmir, Bagh: Department of Botany, University of Azad Jammu and Kashmir, Muzaffarabad. Email: drasiaajk@gmail.com*
2. *Department of Botany, Mohi-ud-din Islamic university Nerian sharif AJK ,Pakistan. Email: s.ashraf084@gmail.com*
3. *Department of Botany, Women University of Azad Jammu and Kashmir, Bagh Email: noshiuajk@gmail.com*
4. *Department of Botany, University of Azad Jammu and Kashmir Muzaffarabad. Email: sidraqayum1@gmail.com*
5. *Department of Chemistry, Hazara University Mansehra, 21300-Mansehra, Pakistan. Email: masabsaeed@hu.edu.pk*
6. *Department of Chemistry, Hazara University Mansehra, 21300-Mansehra, Pakistan. Email: ridazameer04@gmail.com.*
7. *Department of Microbiology, Hazara University, Mansehra-21300, Pakistan. hifsa1arif@gmail.com*
8. *M.Phil Scholar, Department of Botany, Hazara University, Mansehra-21300, Pakistan. Email: azharshahkazmi@hu.edu.pk*
9. *M.Phil. Department of Microbiology, Hazara University, Mansehra-21300, Pakistan. asim.shahzad1992@gmail.com*

Abstract

This study investigates the potential of rhizospheric bacteria isolated from wheat plants in Bajaur, Khyber Pakhtunkhwa, Pakistan, for promoting maize growth. A total of 12 bacterial strains, including *Pseudomonas canadensis* (RW-2), *Enterobacter ludwigii* (RW-5), and *Bacillus aerius* (RW-12), were isolated from the rhizosphere, rhizoplane, and endophytic regions of wheat plants. These strains were characterized based on their morphological and biochemical properties, including phosphate and potassium solubilization, catalase activity, nitrogen fixation, and indole acetic acid production. The bacterial strains were further tested for their compatibility in a consortium and their effect on maize growth in a greenhouse experiment. The results showed that Pseudomonas canadensis and Bacillus aerius exhibited significant phosphate and potassium solubilization activity, while *Enterobacter ludwigii* was effective in producing IAA. The greenhouse experiment revealed that the application of bacterial consortia combined with 90% potassium increased maize plant height by 25%, root length by 42%, and leaf area by up to 48% compared to control treatments. The results suggest that the isolated rhizospheric bacteria significantly enhanced maize growth, offering a sustainable alternative to chemical fertilizers.

Keywords: Rhizospheric bacteria, Plant growth promotion, Phosphate solubilization, Potassium solubilization, Maize growth.



Schematic Abstract

I. Introduction

Soil health is a critical factor for the sustainable growth of crops and the overall agricultural ecosystem. Among the various factors that contribute to soil fertility, rhizospheric bacteria play a vital role in enhancing nutrient availability, promoting plant growth, and improving soil structure (Bashan, de-Bashan, & Hernandez, 2013). The rhizosphere, a narrow zone surrounding the plant roots, is a dynamic and biologically active area where interactions between soil microorganisms and plant roots occur. These interactions include beneficial microbial activities such as nitrogen fixation, phosphate solubilization, and production of growth-promoting substances like indole acetic acid (IAA), which are essential for plant growth and productivity (Bashan et al., 2014; Bhattacharyya & Jha, 2012). The application of plant growth-promoting rhizobacteria (PGPR) has been recognized as a promising alternative to chemical fertilizers, which pose risks to the environment, soil health, and human health (Chauhan et al., 2019; Bhattacharyya & Jha, 2012). The role of rhizospheric bacteria in enhancing nutrient cycling and plant growth is well-documented. Among the various bacterial genera, Pseudomonas, Bacillus, and Enterobacter have been widely studied for their ability to promote plant growth through various mechanisms (Compant et al., 2005; Glick, 2012). These bacteria can solubilize essential nutrients such as phosphorus and potassium, which are otherwise unavailable to plants due to their low soil solubility. Phosphate-solubilizing bacteria (PSB) release organic acids that convert insoluble phosphorus compounds into soluble forms, thus making them accessible to plants (Gupta et al., 2014). Similarly, potassium-solubilizing bacteria (KSB) can release potassium from minerals, providing a crucial nutrient to plants, especially in potassium-deficient soils (Kumar et al., 2017). Additionally, PGPRs are known to produce phytohormones like IAA, which regulate plant growth by influencing root development and

enhancing the plant's ability to absorb water and nutrients (Lal, 2019; Glick, 2012). The positive effects of IAA-producing bacteria have been shown to enhance seed germination, root elongation, and overall plant biomass (Mohite, 2013). Furthermore, PGPRs can induce systemic resistance in plants, making them more resilient to biotic and abiotic stresses such as diseases and drought (Todorova & Kozhuharova, 2010; Pham et al., 2017). These beneficial microbes are thus crucial for improving plant health, increasing agricultural productivity, and reducing the reliance on synthetic fertilizers (Prasad & Babu, 2017; Majeed et al., 2015).

In recent years, the use of PGPRs in agriculture has gained significant attention due to the increasing need for sustainable farming practices. Rhizospheric bacteria not only promote plant growth but also improve soil structure and microbial diversity, leading to long-term soil health benefits (Yadav et al., 2015). Their ability to form symbiotic relationships with plants and facilitate nutrient uptake makes them an important component of integrated nutrient management strategies (Sundar et al., 2019). Furthermore, the use of PGPRs can contribute to reducing environmental pollution by minimizing the need for chemical fertilizers and pesticides, which often lead to soil degradation, water contamination, and greenhouse gas emissions (Thakur et al., 2020). The Bajaur region in Khyber Pakhtunkhwa (KPK), Pakistan, is an agricultural area where wheat is a major crop. However, the soil fertility in this region is often limited by nutrient deficiencies, particularly phosphorus and potassium. These limitations affect crop yield and quality, leading to reduced productivity. Rhizospheric bacteria have the potential to alleviate these nutrient deficiencies by solubilizing phosphate and potassium, thereby improving soil fertility and enhancing crop growth. In this context, the isolation and characterization of rhizospheric bacteria from wheat plants grown in Bajaur could provide valuable insights into the role of PGPRs in promoting maize growth and improving soil health (Singh et al., 2017). In the present study, bacterial strains were isolated from the rhizosphere, rhizoplane, and endophytic regions of wheat plants grown in Bajaur. These bacterial strains were identified based on their morphological and biochemical characteristics, including their ability to solubilize phosphate and potassium, produce IAA, and fix nitrogen. The selected strains were further tested for their compatibility in a bacterial consortium and their effects on maize growth in greenhouse experiments. The aim of this research was to evaluate the potential of these isolated rhizospheric bacteria in promoting maize growth and improving soil nutrient availability, with a focus on phosphate and potassium solubilization (Kumar et al., 2012).

Several studies have reported the potential of PGPRs in enhancing plant growth under controlled conditions. For example, a study demonstrated that phosphate-solubilizing Pseudomonas strains significantly improved maize growth by increasing phosphorus availability in the soil (Gupta et al., 2014). Similarly, Bacillus species have been shown to enhance plant growth by solubilizing phosphorus and producing plant growth-promoting substances (Parmar & Sindhu, 2013). The combination of PGPRs and potassium-solubilizing bacteria has also been shown to improve the growth of various crops, including maize (Yingdui, 2019). The use of PGPRs as biofertilizers is a promising strategy to reduce the environmental impact of conventional fertilizers while improving soil health and crop productivity (Majeed et al., 2015). Moreover, the synergistic effects of bacterial consortia, where different bacterial strains work together to promote plant growth, have been extensively studied (Singh et al., 2018). In this study, the effects of bacterial consortia containing Pseudomonas canadensis, Enterobacter ludwigii, and Bacillus aerius on maize growth were evaluated, with a focus on the impact of potassium and phosphorus solubilization (Zhao et al., 2019). The results of this study could contribute to the development of sustainable agricultural practices in Bajaur and other similar regions with nutrient-deficient soils. By enhancing the availability of essential nutrients through the use of

rhizospheric bacteria, this research could help reduce the dependence on chemical fertilizers, improve soil health, and increase crop productivity. Furthermore, the findings could provide valuable insights into the potential of PGPRs as biofertilizers for maize and other crops, offering an eco-friendly solution to improve food security in developing regions (Bashan et al., 2013).

2. Novelty, Research Gap and Significance of Study

This study focuses on isolating and characterizing rhizospheric bacteria from wheat plants grown in the nutrient-deficient soils of Bajaur, Khyber Pakhtunkhwa, Pakistan, a region with limited research on plant growth-promoting rhizobacteria (PGPR). The study uniquely investigates the synergistic effects of bacterial consortia, combining Pseudomonas, Enterobacter, and Bacillus strains for enhanced phosphorus and potassium solubilization, which is crucial for improving soil fertility. While PGPRs have been widely studied, there is limited research on the specific bacterial strains native to nutrient-deficient regions like Bajaur, and how their interactions in consortia affect crop growth and soil health. This research could significantly contribute to sustainable agricultural practices by promoting the use of PGPRs as biofertilizers. It offers an eco-friendly alternative to chemical fertilizers, improving soil health, reducing environmental degradation, and enhancing crop productivity in areas with nutrient-poor soils, thus addressing food security challenges in developing regions.

3. Materials and Methods

3.1 Bacterial isolation and characterization

In the current investigation, the irrigated land soil of Bajaur bacteria was isolated. After that, the isolated bacteria were checked on maize for their potentiality for the development of maize. Fourteen different places of Bajaur area soil samples were collected. In plastic bag samples individually placed and then only one soil sample was selected. selected sample as Wheat (*Triticumaestivum* L.). The 35 days old plant was used for this research work, from the wheat field plant and was randomly uprooted. Soil samples would be collected from the rhizosphere, and endorhizoplane of the root surface, the separation of the bacteria further examined. Rhizosphere by following the serial dilution agar plate method of Karnwal (2012). To do so, one g wheat rhizospheric soil was aseptically collected and put 1g soil and transferred to 9 ml sterilized distilled water with proper mixing on a rotary shaker for 5 min (Kumar *et al.* 2012) filtered this mixture, label the test tubes, formula 10-1, 10-2, 10-3, 10-4, 10-5,10-6,10-7, 10-8, 10-9, each test tube put 9ml distilled water and then through pipped 1 ml of dilated solution was transferred from 10-1 dilation test tube to10-2. Bacteria were isolated from 10-1 ,10-3 .10-5,10-6 ,10-8. In a pour plat, a small amount (30um inoculum) from a broth culture is added by pipet to the center of a Petri dish. A small volume (0.01ml) of a diluted bacterial mixture containing 100 to 200 cells or less is transferred to the center of LB (Luria Bertani) ager plate and is spread evenly over the surface with a sterile L-shape glass rod and LB nutrient agar plates. From the spreading developed bacterial colony was picked through the sterilized loop and streaked for the purification on LB agar medium plate for further study i.e morphological and chemical study (Gopala 1967) Identification of isolates of bacterial strains Kumar (2013) protocol followed. The procedure for the identification and

isolation of bacterial strains is following. Isolation of bacteria performed from the rhizosphere, by taking one gram of soil from collected samples, and from endophytic and rhizoplane. Samples isolation by roots surface and from roots paste diluted in 9ml sterilized water and (100µl) attenuation utilized to inoculate on LB agar medium. For culturing the inoculated plates will be incubated for at least 2-3 days at 30-36°C. The colony morphology of each isolate was examined on LB agar plates. After 3 days of incubation, different characteristics of colonies such as shape, size, elevation, surface, margin, color, optical characteristics, pigmentation, recorded. For isolated bacteria strains' biochemical characterization, for the checking of biochemical characterization of the isolated bacterial potential the following activities were performed, such as Phosphate solubilization, Bacterial compatibility, Catalase activity, Antibiotic resistance, the following protocol will be followed (Gordon and Weber,1951). A single colony of bacterial culture was and grown on Luria Bertani medium were placed into Pikovskaia's medium containing tricalcium phosphate (Pikovskaia 1948) and incubated at 30 +18C for 4- 5 days. The plates were observed for clear zone formation around the colonies. The zone formation indicated the bacterial strains phosphate solubilize (Gupta *et al.*, 1994). In qualitative media of phosphate solubilizing. For Qualitatively solubilize zone formation the plates have placed an incubator for incubation at 30±1 0C for 5 days and observed formed solubilization zone. Solubilization index (SI) was measured through whole diameter (colony + halo zone) and colony diameter (Premono *et al* 1996). Containing 1000 ml of Pikovskaia.

Bacterial Culture colony was picked up sterilizes loop and transferred to clean glass slide and H2O2 3% 2 drops was added on bacteria colony bubbles was formed shown catalase-positive when bubbles were not formed indicated catalase negative (Singh *et al* 2017). Isolated colonies were grown in nitrogen-free malate media for 48 hours at 28◦c and then transferred to a glass tube and. glass tube head tight with rubber airtight the air removed through injection and acetylene was injected, amount air was removed as equal to acetylene was added. Through Poropak-R column and a flame ionization of the gas chromatograph were shown nitrogenize activity (Hardy *et al.,* 1973).

The production of indole acetic acid was performed by Patten and Glick (1996). The cultured bacteria colony was put in DF (Dworkin and Foster) medium and incubated for 46 hours at 29°C. Strains were isolated after incubation through centrifugation at 4000 rpm for 21 minutes at 3°C (Karnwal 2009). One ml media with strains was mixed in Salkowski's reagent 4 ml and then incubated at moderate temperature for25 minutes. In 25 minutes that's exposed the indole production. The antimicrobial activity of selected bacterial strains was performed on LB agar media by using the protocol Mehmood *et al.*, (1999). 5 mm diameter Sterilized disc paper was dipped in antibiotic and then discs were put in Petri plates (bacterial suspension). The distance between the discs and the edge was 1.5 cm. After 24 hours of incubation at 40˚C, an inhibition zone was formed against the antibiotics. Inhibited zones were measured. (Nikam *et al.*, 2007). Wheat isolated bacteria were checked out the potassium dissolution by followed the spot test method (Parmar and Sindhu 2013) this activity was performed in Aleksandrovsk media. media was prepared and then autoclaved for 48 minutes and then carefully put in Petri plats under the laminar flow. Media were solidified, bacterial strain suspension was prepared and 0.5cm in diameter disc dipped in bacterial suspension and this disc put in mid of the Aleksandrovsk media plates. the plate was put in an incubator for 3 days at 38˚C. Zones formed after 3 days were measured on a simple ruler. Compatibility of the strains selected for the bacterial consortium Bacteria was grown individually and together in LB broth liquid medium at 27°C and 180 rpm for 72 hours. Therefore, three compatible strains were selected for the bacterial consortium, cultured bacterial strain in LB

broth liquid medium 28ul was spread in each plate of LB agar media. In that's plates wells formed through ager well borer, wells formed the same size, cultured bacterial strain in LB broth liquid medium was poured in this wells bacteria cultured other than which was spread in this wells and incubated at 40C for 24 hours after incubated cheeked the zones based on antagonistic and synergistic effects of bacterial strains. Compatibility of the strains selected for the bacterial consortium.

3.2 Green house experiment

A greenhouse experiment was performed to check the effect of rhizospheric bacteria and potassium in maize plants. For the greenhouse experiment, 32 pots were prepared, each pot containing 7kg soil having 3:1 sand and clay total of 8 treatments were made in the greenhouse experiment, for each treatment has three replicates were made and added recommended fertilizer, i.e DAP (Di-ammonium phosphate), urea, and potassium, potassium in different percentage, InT1and T230%, T3 and T4 60%, T5, and T6 90% and T7 and T8 120% and rhizospheric bacteria added in T230%, T4 60%, T6 90% and T8 120% potassium. A Maize Pahari variety seeds were used; Pahari variety was collected from the Buffa research centre of Mansehra. A germination test was performed to check the viability of seeds; seeds were kept in petri plates. water and filter paper was placed in Petri plates for the germination of seeds. The seeds were soaked for 4-5 days. Seeds were germinated after 5 days and produced radicals. This test was performed by using the following formula Mukhtar (2008).

$$\text{Seed germination (\%)} = \frac{\text{Germination seed} \times 100}{\text{Total}}$$

The bacterial strain was grown together in an LB broth liquid medium at 27°C and 180 rpm for 72 hours. seeds sterilized with 70% ethanol and 30% distilled water. After sterilization seeds depth 45 minutes in a consortium for coting and then dried for 35 minutes. Sowing seeds in the morning time and per pot three to four seeds were sowed, each seed was dept 1m (2.5 cm) inch in soil and recommended dose fertilizers were used for each pot. After one-week plants were grown in pots. T1=Recommended Dose Fertilizers (RDF) + Murate of potash (MOP) 30%, T2=RDF+ MOP 30% + consortium, T3=RDF+ MOP 60%, T4=RDF+ MOP 60% +consortium, T5= RDF+MOP 90%, T6=RDF+ MOP 90% + consortium, T7=RDF+MOP 120% and T8=RDF+MOP 120% + consortium. Maize plant height, plant thickness, the total number of leaves, stem color, the colour of leaves, smallest leave height, largest leave height, smallest leaves size, largest leave size, root length, root fresh weight, shoot fresh weight was measured each plant

3.3 Statistical analysis

The statistical analysis of the results was done through *Statistics* 8.1 as well as the mean values were evaluated by the method of Steel and Torrie, 1960.

4. Results

4.I Isolated Rhizobacteria from wheat

Wheat samples with rhizosphere soil were collected from Bajaur (Khar). Total 12 strains (RW1, RW2, RW3, RW4, RW5, RW6, RW7, RW8, RW9, RW10, RW11, RW12) were isolated from wheat rhizosphere, rhizoplane, and endophytic on agar media. Three strains (RW2, RW5, RW12) were selected for the growth of maize-based on phosphate and potassium solubilization potential.

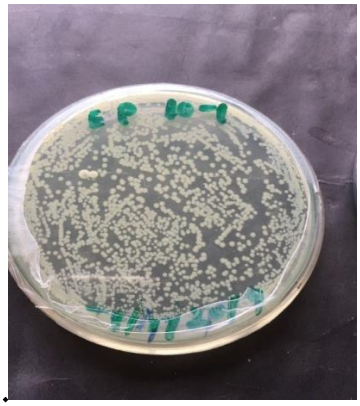


Figure 1: Different bacterial colonies culture on L.B agar medium

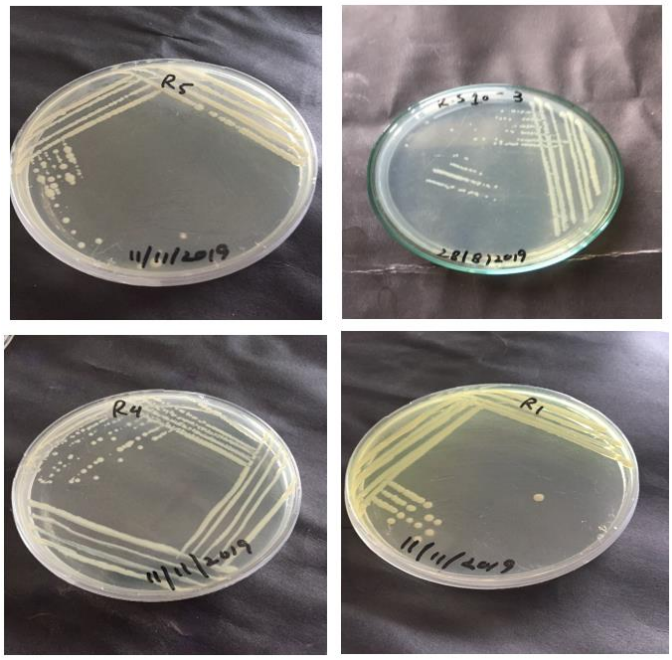


Figure 2: Bacterial colony streak

4.I.I Morphology of isolated strains

Wheat rhizospheric isolated bacterial strains were studied under an electronic microscope. Isolated bacterial strains show different morphological features i.e color, off-white to transparent and white. colonies shape as mostly circular and cell shape as mostly rod shape. colonies margin as mostly nodule and even. Colony elevation is convex to raised and convey (Table 1).

Table I Bacterial colony morphological properties on the basis of isolation from sample.

Isolatea name	Isolates sources	Colony form	Color of colony	Colony margins	Cell shape	Elevation of Colony
RW-2	Rhizosphere	Circular	Off-white	Nodulate	Rod	Convex
RW-5	Rhizoplane	Punctiform	Transparent	Nodulate	Rod	Convex
RW-12	Endophytic	Circular	White	Even	Rod	Raised

4.1.2 Identification of isolated strains

Wheat isolated bacterial strains were identified through gene sequence BLAST 16S-rRNA gene sequence analysis (Table 2).

Table 2 Molecular identification of isolated bacterial strains

Bacterial strains name	Length of sequence (bp)	Accession number	Name of species	Accession	Similarity
RW-2	1017	MT002745	*Pseudomonas canadensis*	NR156852.1	99.88%
RW-5	968	MT114433	*Enterobacter ludwigii*	NR042349.1	99.88%
RW-12	1042	MT114435	*Bacillus aerius*	NR118439.1	99.46%

4.2 Important traits of isolated Rhizobacteria

4.2.1 Phosphate solubilization

Three isolated bacterial strains from the Wheat rhizosphere, rhizoplane, and endophytic, Isolated bacterial strains were tested in Pikovskaia media. Among 3 isolated bacterial strains *Bacillus aerius sp* (RW-12) 1.1cm showed clear zones of phosphate solubilized after four days, *Pseudomonas Canadensis sp* (RW-2) made 1.6 zones, and *Enterobacter ludwigii sp* (RW-5) not formed zone after four days. *Pseudomonas Canadensis sp* (RW-2) zone greater than *Bacillus aerius sp* (RW-12).

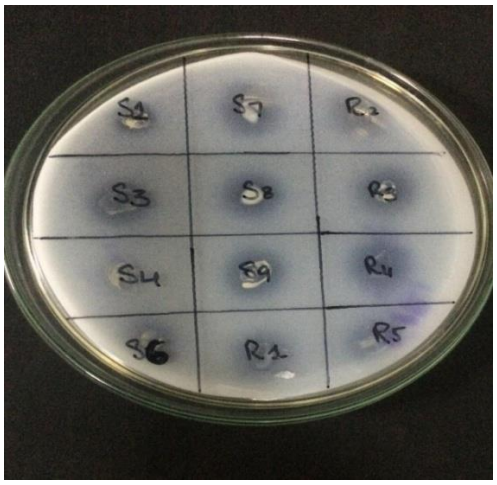


Figure 3: Phosphate solubilization of bacterial strains

4.2.2 Catalase activity

Catalase test of bacteria single colony produce bubbles on hydrogen peroxide drops was considered as positive. *Pseudomonas Canadensis sp* and *Enterobacter ludwigii sp* as positive and *Bacillus aerius* sp are negative bubbles were not formed (Table 3).

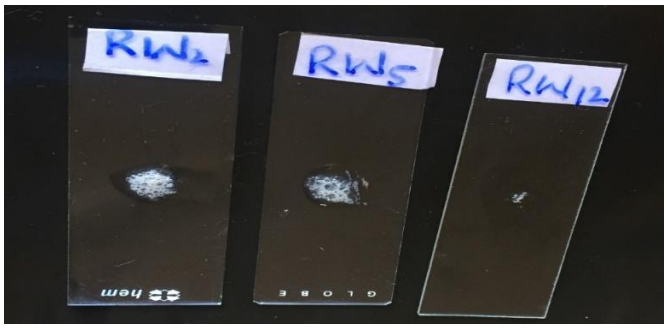


Figure 4: Catalase test of the strains.

Table 3 Catalase test of isolated bacterial strains

Bacterial strains names	Bacterial species name	Catalase positive	Catalase negative
RW-2	*Pseudomonas Canadensis sp*	+	0
RW-5	*Enterobacter ludwigii sp*	+	0
RW-12	*Bacillus aerius sp*	0	+

4.2.3 Nitrogenize activity

isolates strains nitrogenase activity was performed. *Pseudomonas Canadensis sp* as nitrogen fixation positive effect and *Enterobacter ludwigii sp, Bacillus aerius sp* as nitrogen fixation negative (Table.4.5).

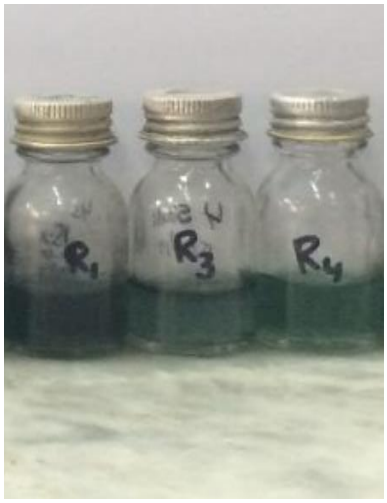


Figure 5: Nitrogenize test of the strains

4.2.4 Indole acetic acid production

Indole acetic acid activity of the *Pseudomonas Canadensis sp*, *Enterobacter ludwigii sp, Bacillus aerius sp* was tested. These bacteria were produce IAA. All Bacteria strains were shown a positive effect in indole acetic acid (Table.4.5).

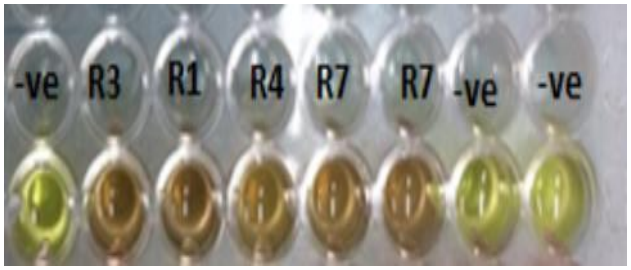


Figure 6: Indole acetic acid test of the bacterial strains.

4.2.5 Antibiotic activity of isolated bacteria strains

Isolated bacteria RW2, RW5, RW12 (*Enterobacter ludwigii sp*, as *Pseudomonas Canadensis sp*, *Bacillus aerius sp*) tested in different antibiotics such as amoxicillin, Vibramycin, and erythromycin in the same concentration (Table 4.6).

4.2.5.1 Effect of amoxicillin (amoxil)

Wheat isolated bacteria selected for antimicrobial activity, bacteria show resistance against antibiotics. The concentration of amoxicillin was 1mg/ml and disk size was 5mm, RW2 (*Pseudomonas Canadensis sp)* shown resistance against antibiotic amoxicillin and made the highest zone 4.5cm and *Enterobacter ludwigii sp* made the lowest zone 2.5 cm. RW12 *(Bacillus aerius sp)* not formed zones against antibiotics (Table 4.4).

4.2.5.2 Vibramycin

Isolated bacteria were tested in Vibramycin. The concentration of Vibramycin is 1mg/ml. *Pseudomonas Canadensis sp* showed resistance 3.6cm zone against the Vibramycin, RW5 (*Enterobacter ludwigii sp)* 2.2cm zone formed, RW12 *(Bacillus aerius sp)* 1cm inhibition zone was formed and resistance against the Vibramycin antibiotic (Table 4.4).

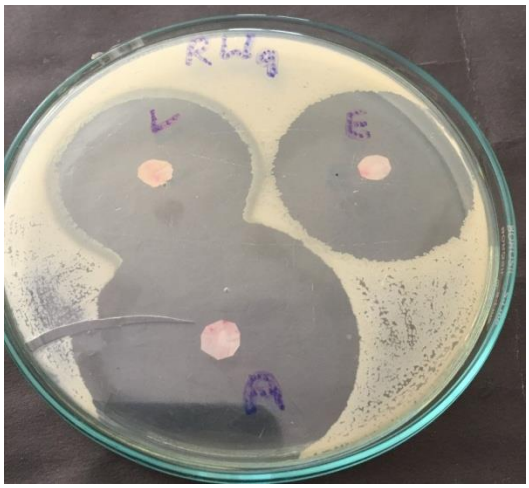


Figure 7: Antibiotic activity of bacterial strains

4.2.5.3 Erythromycin

Isolated bacterial strains were tested in erythromycin antibiotics. Antibiotic concentration same to Vibramycin, RW2 *Pseudomonas Canadensis sp* shown resistance against erythromycin and made 3.8cm zone, RW5 (*Enterobacter ludwigii sp)* was observed resistance against erythromycin, the lowest 1cm zone was examined. RW12 (*Bacillus aerius sp)* 1.1cm resistance zone was observed and resist against the erythromycin (Table 4).

Table 4 Antibiotics applied on isolated bacterial strains

Bacterial strains	Name of bacteria	Amoxicillin	Erythromycin	Vibramycin
RW-2	*Pseudomonas Canadensis sp*	Resistant	Resistant	Resistant

RW-5	*Enterobacter ludwigii sp*	Resistant	Resistant	Not resistant
RW-12	*Bacillus aerius sp*	Not resistant	Resistant	Resistant

4.2.6 Potassium solubilization activity

Strains selected for potassium solubilization activity, all strains were shown potassium solubilized. *Pseudomonas Canadensis sp* was shown 1.4 cm zone and *Enterobacter ludwigii sp* made 2.6 cm zone was formed and, *Bacillus aerius sp* was formed 2 cm zone (Table.5).

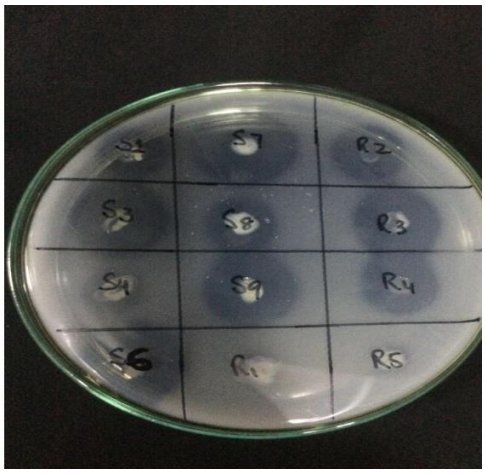


Figure 8: Potassium solubilizing test of the bacterial strains

Table. 5 Indole acetic acid, Nitrogen fixation and potassium solubilization activity of isolated strains.

Strains name	IAA effect	Nitrogen fixation	Potassium solubilization
RW2*(Pseudomonas Canadensis sp)*	Positive	Positive effect	Positive
RW5*(Enterobacter ludwigii sp)*	Positive	Negative	Positive
RW12*(Bacillus aerius sp)*	Positive	Negative	Positive

4.2.7 Compatibility of bacterial strains

Wheat isolated bacteria strains applied on maize Pahari verity, applied bacteria as RW5 *(Enterobacter ludwigii sp),* RW12 *(Bacillus aerius sp)* and RW2 (*Pseudomonas Canadensis sp)* showed synergetic effect

and to each other before maize (Pahari verity) sowing in a greenhouse experiment to check the compatibility of bacterial strains (Table 6).

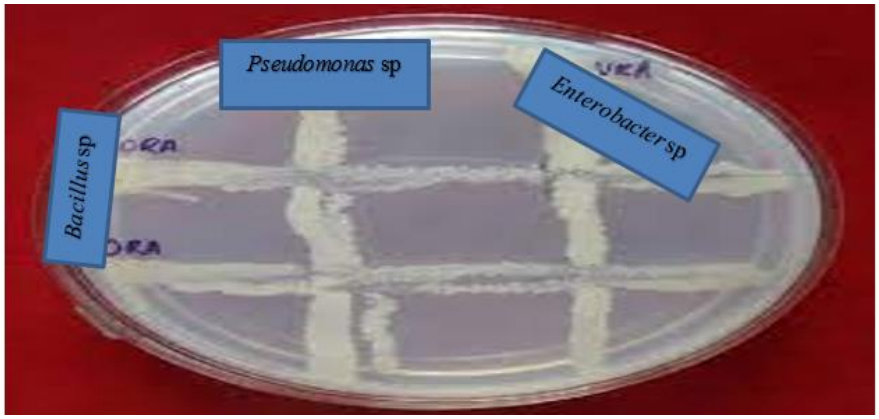


Figure 9: Bacteria compatibility test

Table 6 Compatibility effect of bacterial isolated strains

Bacterial strains	Name of bacteria	Compatibility effect of bacteria
RW-2	*Pseudomonas canadensis sp*	Synergetic
RW-5	*Enterobacter ludwigii sp*	Synergetic
RW-12	*Bacillus aerius sp*	Synergetic

4.3 Green house pot experiment

4.3.1 Plant height (cm)

In the greenhouse pots experiment plant height of treatment number three 25% is greater than consortium (RW2, RW5, RW12) (*Pseudomonas Canadensis sp + Enterobacter ludwigii sp*+ *Bacillus aerius sp*) treatment number two. Potassium concentration as 60% in treatment number three and concentration of potassium 30% treatment number two, plant height of treatment number three having 60% potassium 13% as greater than treatment number one having potassium 30%, plant height showed no significant result (Table 4.7).

4.3.2 Thickness (girth) of plant (cm)

Plant thickness of consortium 23% as greater than control, concentration of potassium as 90% in consortium and 30% potassium concentration in control, plant thickness shows the significant result. RW2, RW5, RW12 *(Pseudomonas canadensis sp + Enterobacter ludwigii sp+ Bacillus aerius sp*) + RDF (consortium) 6% increase over the control (Table 7).

Table 7 Different growth parameters of maize plant

Treatments	Plant height (cm)	Plant thickness (grith) (cm)
Murate of potash(MOP) 30% + urea+DAP	52.3AB (±5.79)	13.0A (±3.08)
Murate of potash(MOP) 30%+urea+DAP + (consortium)	47.0B (±11.26)	13.7A (±1.44)
Murate of potash(MOP) 60% +urea+DAP	59.6A (±1.97)	16.1A (±1.65)
Murate of potash(MOP) 60% +urea+DAP + (consortium)	58.6AB (±8.51)	15.5A (±3.24)
Murate of potash(MOP) 90% +urea +Diammonium phosphate	49.0AB (±20.50)	15.0A (±4.08)
Murate of potash(MOP) 90%+urea+DAP + (consortium)	50.8AB (±13.15)	16.8A (±6.25)
Murate of potash(MOP) 120%+urea+DAP	53.0AB (±6.78)	15.6A (±3.14)
Murate of potash(MOP) 120%+urea+DAP + (consortium)	58.8 AB (±8.77)	15.7A (±2.98)

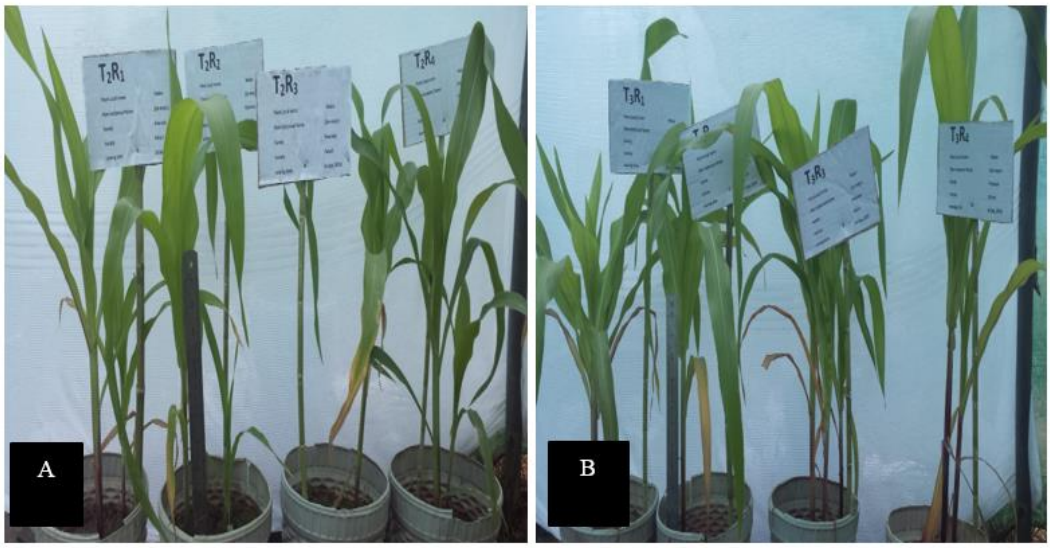


Figure 10: Comparison of plant height between treatments 30% (A) and 60%(B) potassium

4.3.3 Number of leaves

In greenhouse pots experiment plant growing, consortium + RDF treatments growth grater 33% than control treatments. In consortium treatments number of leaves showed significant results in greenhouse experiment (Table).

Table 8 Various treatment show deferent results in maize leave number.

Treatments	Number of leave
Murate of potash(MOP) 30% +urea+Diammonium phosphate	6.2C (±0.95)
Murate of potash(MOP) 30% +urea +Diammonium phosphate +(consortium)	7.0ABC (±0)
Murate of potash(MOP) 30% +urea (0.556g) +Diammonium phosphate	7.5AB (±0.57)
Murate of potash(MOP) 30%+urea +Diammonium phosphate+ (consortium)	7.2ABC (±0.95)
Murate of potash(MOP) 30% +urea +Diammonium phosphate	7.0ABC (±0.81)
Murate of potash(MOP) 30%+urea +Diammonium phosphate+ (consortium)	8.0A (±0.81)
Murate of potash(MOP) 30% +urea +Diammonium phosphate	6.7BC (±0.5)
Murate of potash(MOP) 30%+urea +Diammonium phosphate + (consortium)	7.2ABC (±0.95)

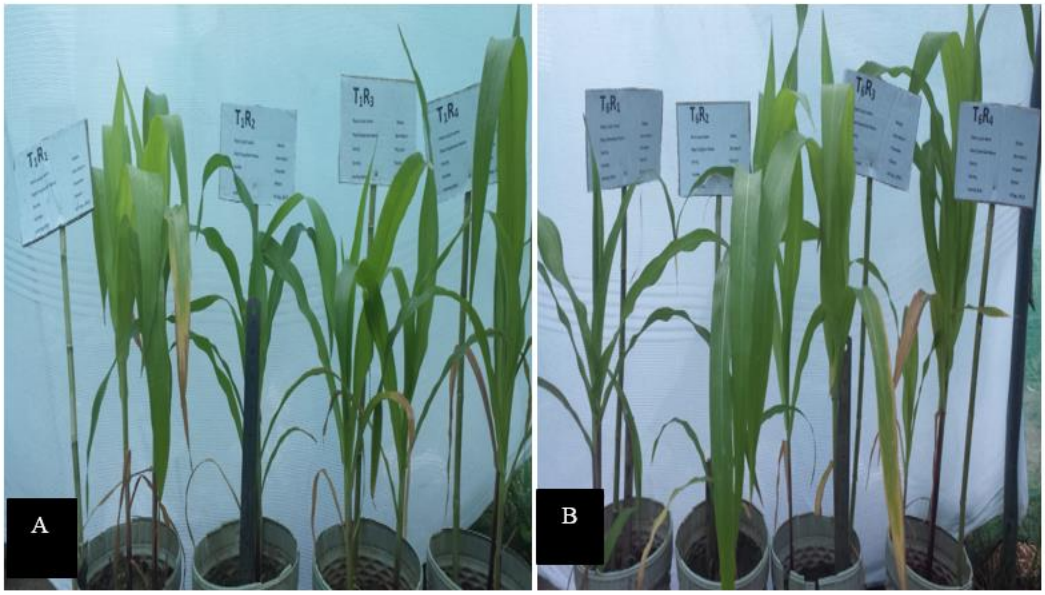


Figure 11: Comparesin between 30% (A) and 60% (B) potassium

4.3.4 Largest leave length (cm)

In treatment number three 90% potassium concentration, Consortium + RDF leave length 48% more as compared with other consortium +RDF and 30% potassium concentration result, shown significant results (Table 4.9).

4.3.5 Smallest leave length(cm)

The smallest leave length of maize plant in greenhouse experiment, treatment number three consortium +RDF + 90% concentration of K 187% increase leave length than treatment four 120% potassium concentration and consortium, shown significant results (Table 9).

Table 9 Different growth parameters of maize leaves

Treatment	Largest leave length (cm)	Smallest leave length (cm)
MOP 30%+ DAP + Urea	42.4B (±7.85)	10.2A (±3.45)
MOP 30%+ DAP+ Urea+ consortium	38.8B (±6.27)	5.7C (±2.24)
MOP 60% + DAP + Urea	45.3AB (±11.17)	9.8AB (±4.58)
MOP 60% + DAP + Urea + consortium	48.8AB (±3.17)	6.5BC (±2.61)
MOP 90%+ DAP + Urea	41.0B (±10.65)	5.62C (±1.25)

MOP 90% + DAP + Urea + consortium	56.6A (±7.44)	11.2A (±1.44)
MOP 120% + DAP + Urea	51.3AB (±12.39)	5.8C (±2.12)
MOP 120% + DAP + Urea + consortium	49.3AB (±12.63)	3.90C (±0.2)

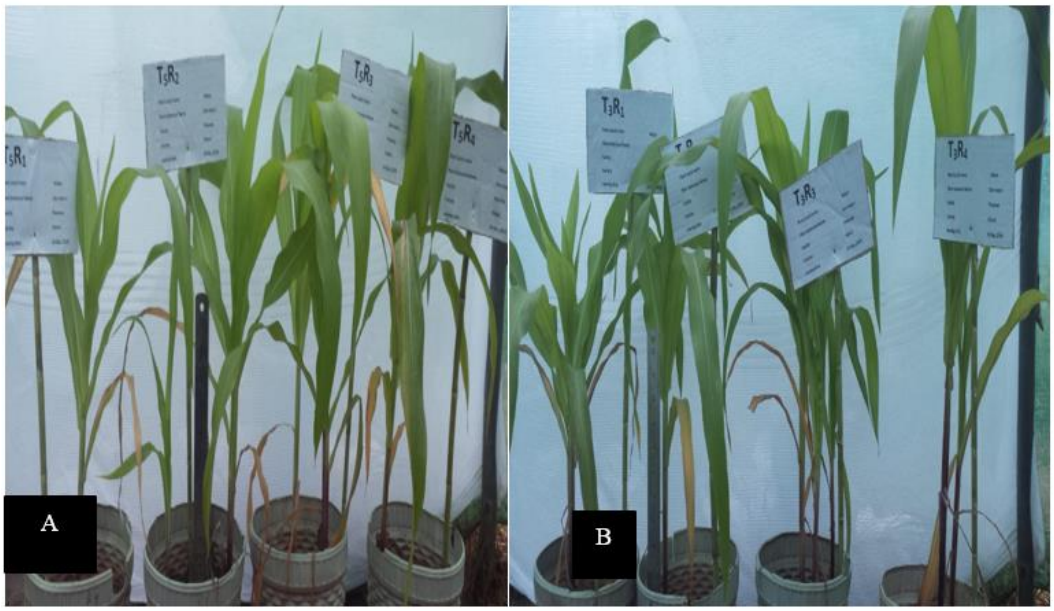


Figure 12: Comparesin between 60% (A) and 90% (B) potassium

4.3.6 Largest leave size (cm)

Leave size in treatment number one having potassium 30% and *Pseudomonas Canadensis sp + Enterobacter ludwigii sp+ Bacillus aerius sp* (consortium) were also added as less than control treatment number four having 120% potassium. control 41% more than a consortium.120% potassium treatment shown significant result. (Table 4.10).

4.3.7 Smallest leave size(cm)

The smallest leaf size was recorded in treatment number four 73% having 120% MOP+ DAP+ Urea+ consortium as highest than normal treatment number two having 60% potassium. 120% potassium concentration of consortium in treatment number four and 60% potassium in control treatment number two. Shown significant result. (Table 10).

Table 10 Different growth parameters of maize leaves

Treatment	Largest leave size (cm)	Smallest leave size (cm)

MOP 30%+ DAP+ Urea	43.0A (±1.87)	15.1AB (±4.32)
MOP 30%+ DAP+ Urea+ consortium	39.0AB (±7.63)	11.0B (±3.08)
MOP 60%+ DAP+ Urea	45.6A (±5.79)	9.8B (±2.25)
MOP 60%+ DAP+ Urea+ consortium	46.3A (±9.13)	11.6AB (±4.88)
MOP 90%+ DAP+ Urea	44.3A (±4.26)	12.6AB (±6.60)
MOP 90%+ DAP+ Urea+ consortium	39.6AB (±5.51)	11.50AB (±2.54)
MOP 120%+ DAP+ Urea	46.8A (±6.25)	11.7A (±6.29)
MOP 120%+ DAP+ Urea+ consortium	33.2B (±2.13)	17.0AB (±8.86)

4.3.8 Shoot weight (mg)

Shoot weight 75% increase of the control treatment number three having 90% K concentration as compared to 30%potassium concentration of control treatment number one.

4.3.9 Root length (cm)

Treatment number one having 30%potassium concentration as compared to treatment number two having a potassium concentration of 60%, root length of treatment number two is 42% greater as compared to treatment number one (Table 4.11).

4.3.10 Root weight(mg)

Treatment number one as compared to treatment number two, treatment number one root weight 53% as more than treatment no. two in a greenhouse experiment. two number treatments consist of 60% potash and one number treatment having 30% potash (Table 11).

Table 11 Different growth parameters of maize plant

Treatment	Shoot weight (mg)	Root length (cm)	Root weight (mg)
MOP 30%+ DAP+ Urea	25.5B (±7.08)	19.6D (±0.85)	3.2A (±0.95)
MOP 30%+ DAP+ Urea+ consortium	28.8AB (±3.68)	19.5D (±2.97)	3.0A (±0.81)
MOP 60%+ DAP+ Urea	37.7AB (±6.84)	27.8A (±4.21)	4.0A (±1.41)
MOP 60%+ DAP+ Urea+ consortium	42.8AB (±19.02)	26.1ABC (±4.06)	4.6A (±1.65)
MOP 90%+ DAP+ Urea	44.8A (±15.36)	21.8BCD (±5.89)	3.9A (±1.54)
MOP 90%+ DAP+ Urea+ consortium	37.8AB (±7.04)	21.4BCD (±2.33)	3.9A (±0.77)
MOP 120%+ DAP+ Urea	35.2AB (±14.43)	26.4AB (±6.25)	4.6A (±1.47)
MOP 120%+ DAP+ Urea+ consortium	28.8AB (±17.88)	20.5CD (±2.64)	4.12A (±2.17)

Figure 13: Comparesin between 30% (A) and 60% (B) potassium#

Figure 14: Comparison between 30% (A) and 60% (B) potassium

5. DISCUSSION

The Rhizospheric bacteria were isolated from the rhizosphere, rhizoplane, and endophytic of wheat crop grown up in the plain area of Bajaur region KPK (Khyber Pakhton Khwa) in Pakistan. strain *Bacillus aerius* (RW-12) *Pseudomonas canadensis* (RW-2) *Enterobacter ludwigii* (RW-5). Shivaji *et al* (2006)

also isolated *Bacillus* from the air in different altitudes of atmosphere. Bacterial strains are isolated based on physiological and chemical characteristics. Three isolated bacterial strains and potassium promoted plant growth. Potassium played important role in the plant height of maize, plant diameter, root length, and mass (weight). Swetha *et al* (2017) was also added potassium in four different concentrations (0, 30, 60, and 90 kg ha-1) in popcorn (*Zea mays L*). In recent research work checked the phosphate solubilization of *Pseudomonas Canadensis* (RW-2), *Bacillus aerius* (RW-12), *Enterobacter ludwigii* (RW-5). *Pseudomonas canadensis* (RW-2), *Bacillus aerius* (RW-12) shown zones of phosphate solubilization. *Pseudomonas Canadensis* , *Bacillus aerius* phosphate solubilized bacterial strains. Jyothi*et al* (2013) also worked *Psedomonas* sp to find out the phosphate solubilization. Sanjotha and Manawadi (2016) also performed phosphate solubilization activity in *Pseudomonas sp*, *Bacillus sp*, and *Rhizobium* observed high phosphate solubilization. Catalase activity was also performed in this research work, *Enterobacter ludwigii sp* and *Pseudomonas Canadensis* as catalase-positive, and *Bacillus aerius* as catalase-negative. Babiker *et al* (2016) was collected 50 different samples from a different area of Khartoum state for production of catalase, in these samples 29 *Bacillus species*, and out of these twenty-nine twenty shown catalase positive. In recent research work was also performed the nitrogen fixation activity, nitrogen fixation was occurring in *Pseudomonas Canadensis* and *Enterobacter ludwigii, Bacillus aerius* did not occur nitrogen fixation. Pham *et al* (2017) was also isolate *pseudomonas stutzeri* from the rhizosphere of rice and checked out the nitrogen fixation activity, this strain was a nitrogen-fixing strain. *pseudomonas stutzeri* was applied on rice seedling, they shown good results on growth as compared with control (without *pseudomonas stutzeri*) and also compared with chemical nitrogen was added to plant. Potassium activity was performed in this research, all bacterial strains RW2, RW5, RW12 (*Pseudomonas Canadensis* and *Enterobacter ludwigii, Bacillus aerius)* was shown potassium solubilizing bacteria. Yingdui He 2019 also worked on potassium were added to Banana root, potassium was added in a different concentration high potassium, low potassium no potassium, and normal amount of potassium, too high and too low concentration of potassium was not shown an effect on Banana root but changed the gene sequence expressively. That was shown that potassium also changes gene sequence Chen *et al* (2020) was worked on *Monochasma savatieri* to checked the effect of the nitrogen, potassium, and phosphorus in *Monochasma savatieri*. The inorganic element shows significant results in *Monochasma savatieri*. This research work was also performed on the IAA activity, *Pseudomonas Canadensis sp* and *Enterobacter ludwigi, Bacillus aerius*, all were shown a negative effect. Mohite, (2013) 15 bacteria were isolated from rhizospheric soil, ten were more IAA producing bacteria, and five as optimum producing IAA. It was recommended that indole-producing bacteria worked like a biofertilizer to increase plant growth. In this research bacterial compatibility was found out of the *Enterobacter ludwigii* sp and *Pseudomonas Canadensis* and *Bacillus aerius* . *Enterobacter ludwigii sp, Bacillus aerius* sp as an antagonistic effect, and *Pseudomonas Canadensis sp* showed a synergetic effect. Prasad and Babu (2017) were performed worked on bacteria compatible, *Azospirillum brasilense*strain and *Pseudomonas fluorescens* strain, both strain compatible with each other and enhanced plant root, shoot, and increase the number of leaves. In our research work all isolated bacteria, *Enterobacter ludwigii, Pseudomonas Canadensis*, and *Bacillus aerius* were performed antibiotic activity. Antibiotics as amoxicillin, Erythromycin, and Vibramycin, all antibiotics having same concentration 1mg/ml shown different effect against the bacterial species. *Pseudomonas Canadensis* showed more resistance and formed 4.5cm zone against antibiotic Amoxycillin, *Enterobacter ludwigii* shown low resistance 2.5 cm zone formed against the Amoxycillin and RW12 (*Bacillus aerius)* not resist again the amoxicillin 1mg/ml. Effect of Vibramycin in RW2 (*Pseudomonas Canadensis)* shown more resistance 3.6cm zone formed and RW5 *(Enterobacter ludwigii)* (2.2cm zone formed) and *RW12 (Bacillus*

aerius) (1cm zone formed) shown low resistance against Vibramycin. 1mg/ml erythromycin antibiotic against the *Pseudomonas Canadensis sp* shown more effected 3.8cm zone formed (more resisted) than *Bacillus aerius*(1.1cm zone formed) and *Enterobacter ludwigii* (1cm zone). Todorova and Kozhuharova (2010) were isolated *Bacillus* strains from a soil sample and this strain was tested by antibiotic activity that's shown highly antimicrobial activity. In our greenhouse experiment potassium and *Pseudomonas Canadensis, Enterobacter ludwigii, and Bacillus aerius* (consortium) was added to pots (maize plants Pahari variety) they show the best growth results in plants. Majeed *et al* (2015) were also isolated strains AJK-1 AJK-2 AJK-3 AJK-4 AJK-5 AJK-6AJK-7 AJK-8 AJK-9 from the wheat of the Himalayan region of Pakistan, four strains Bacillus species, *Acetobacter pasteurianussp, Stenotrophomonas* species, and *S. rhizophila* sp was applied on wheat in the early stage. it was observed the significant growth shoot and root length and weight. In our greenhouse pots, experiment potassium was added in different percentages 30%, 60%, 90%, and 120%. 90% potassium shown the best results and then 60% and 120%. Maize was shown plant height of control + recommended dose fertilizers + 60% potassium treatment as 25% greater than other control treatment containing 30% potash. This experiment showed the plant diameter of the consortium treatment (*Pseudomonas Canadensis*, *Enterobacter ludwigii, and Bacillus aerius)* 23% increase as compared with control treatment. Karnwal (2017) three rhizospheric bacterial strains isolated *Pseudomonas aeruginosa, P. fluorescens,* and *Bacillus subtilis* isolated from maize plant and applied on rice plant. It was noted the best results of the rhizospheric bacteria in rice plants growth. In this work was also noted the number of leaves in maize plant, treatment containing *Pseudomonas Canadensis*, *Enterobacter ludwigii and Bacillus aerius* and 90% K as 33% more than the control treatment. In consortium treatment area of the maize, leave was expressively affected by *Enterobacter ludwigii, Pseudomonas Canadensis, and Bacillus aerius* and potassium dose, in consortium treatment was noted more leaf area as compared with control (*Enterobacter ludwigii, Pseudomonas Canadensis, and Bacillus aerius* was not added). Herdiyantoro *et al* (2018) also was work on maize potassium solubilizing bacteria, fifteen strain were isolated from the maize rhizosphere, in these fifteen isolated bacteria strain were find out the potassium solubilization, in maize plant, only three strains (RBPK-DHJ3-3150, RBPK-DHJ1-4125, and RBPK-DHJ2-5250 KSRB isolates) were shown potassium solubilizing strains. In our research work was also observed the maize plant stem and root fresh weight, stem weight of maze fresh plant 90% potash was applied as 75% greater than 30% potassium was applied. potassium was 60% applied the root weight of the maze plant was recorded 53% increase than 30% potassium was added. Amanullah et al (2016) were worked on maize to respond to potassium and without potassium. potassium was applied shown best growth and potassium was not added shown weak growth.

Conclusion

Wheat receiving isolated Rhizobacteria from district Bajaur. These strains promoting plant growth and increasing maize plant biomass. These three tested bacteria strain (RW2, RW5, RW12) shown phosphate and potassium solubilized bacteria. Strains were shown to have the ability to catalase-positive, antibiotic resistance, nitrogen fixation. Especially *Pseudomonas Canadensis,* and having the quality to be Icompatible with other bacteria to grow easily. Potassium 60% was added to control the treatment of maize plant, plant height 25% increase than 30% potassium in a greenhouse experiment.RW2, RW5, RW12 (*Enterobacter ludwigii, Bacillus aerius, Pseudomonas Canadensis)* and90% potassium shown maize plant diameter 23% increase leave length 48%-187% and 120% potassium shown 41%-73% increase maize plant leave size. Stem weight 75% more in 90% potassium than 30% potassium. In the control treatment, 60% K was added

to pots 42% greater than 30%. The results showed that isolated bacteria can dissolve agrochemicals. there should be proper awareness for the farmer that the use of the PGPR with chemical fertilizers affects plant growth positively, and can reduce the use of chemical fertilizers.

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