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BIOCHEMICAL ACTIVITIES AND MICROBIAL POPULATION IN SOIL AS INFLUENCED BY PGPR INOCULATION IN A VERTISOL

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ABSTRACT

A field experiment was conducted at research station, JNKVV, Jabalpur (M.P.) during 2023-24. The experiment was designed in randomized block design (RBD) with 10 different treatments. These treatments included 7 PGPR strains, one local isolate of Phosphate Solubilizing Bacteria (PSB), and 2 control treatments: fertilized uninoculated (FUI) and unfertilized uninoculated (UFUI). *Enterobacter asburiae* strain R31 (T_1) significantly improved acid and alkaline phosphatase activities were enhanced to 20.61 (µg PNP hr^{-1} g^{-1} soil) and 29.3 (µg PNP hr^{-1} g^{-1} soil), respectively. Dehydrogenase activity was increased to 6.56 (µg TPF g^{-1} hr^{-1} soil), and urease activity was raised to 7.07 (µg urea hydrolyzed g^{-1} hr^{-1} soil). Additionally, the populations of Rhizobium sp. and PSB sp. were found to be highest in the T_1 treatment, at 7.80 log cfu (63.09 × 10^6 cfu g^{-1} soil) and 8.83 log cfu (67.60 × 10^7 cfu g^{-1} soil), respectively. These increases in enzymatic activity and microbial populations suggest that soil health and fertility were effectively enhanced by *Enterobacter asburiae* strain R31.The potential of this strain as a valuable bio-inoculant for sustainable agriculture.

***Keywords* :** Biochemical activities, microbial population, PGPR, Vertisol.

Introduction

Chickpea (*Cicer arietinum* L.), commonly known as Bengal gram, is recognized as an important winter pulse crop in India. It is classified under the genus *Cicer*, within the sub-family *Papilionaceae* of the family *Leguminosae*. Chickpea is regarded as highly nutritious, as it is composed of significant amounts of protein, carbohydrates, and essential minerals such as phosphorus, calcium, magnesium, iron, and zinc, along with β-carotene. The protein quality of chickpea has been found to be superior compared to that of many other legume crops (Jukanti *et al*., 2012; Siddique *et al*., 2012). Pulses, including chickpea, are considered vital for sustaining agricultural productivity. They are cultivated in diverse cropping systems, and their contribution to soil fertility is ensured through biological nitrogen fixation. Soil porosity is enhanced by their tap root structure (Verma *et al*., 2016). The excessive use of chemical pesticides and fertilizers has been linked to adverse effects on human health, crop quality, and the environment. Over time, soil fertility has been degraded, and pollution levels have increased. As a result, the use of biofertilizers is being promoted as a sustainable and organic alternative to reduce dependence on chemical inputs.Biofertilizers offer a promising solution by providing essential nutrients to plants with a reduced need for inorganic fertilizers, potentially lowering their application by 25–30%, even within the Soil Test Crop Response (STCR) framework (Vance, 1997; Rana *et al.,* 2012).

Plant Growth-Promoting Rhizobacteria (PGPR) strains have been demonstrated to significantly enhance the populations of beneficial microbes, including nitrogen-fixing bacteria and phosphate-solubilizing bacteria (PSB), within the chickpea rhizosphere. Through PGPR inoculation, the microbial diversity and density in the root zone have been increased. As a result, improved nutrient cycling and availability have been observed, particularly for nitrogen and phosphorus.This enhanced microbial activity has been associated with more efficient nutrient uptake by the plant, contributing to better growth and yield (Ahmad *et al*., 2008). In various field studies, the populations of *Rhizobium* and PSB were quantified at harvest. These studies confirmed that symbiotic nitrogen fixation and phosphate solubilization efficiency had been improved. Overall, PGPR application has been recognized as an effective strategy to promote sustainable chickpea production by reducing the need for chemical fertilizers. The enhancement of soil enzymatic activities by PGPR strains can be attributed to several mechanisms.PGPR inoculation increases microbial biomass in the rhizosphere, leading to higher production and activity of soil enzymes (Saharan & Nehra, 2011). PGPR stimulated plants release more root exudates, which serve as substrates for soil microorganisms, thereby enhancing enzymatic activity (Vessey, 2003). It can improve soil aggregation, which promotes a favorable environment for microbial activity and enzyme stability (Bais*et al.,* 2006). The acid and alkaline phosphatage activities were investigated in rhizospheric soil at postharvest of chickpea**.**

Materials and Methods

The field experiment at the Research Farm of the Department of Soil Science & Agricultural Chemistry, JNKVV, Jabalpur (M.P.). The experiment was laid out in a randomized block design (RBD) with 10 different treatments. These treatments included 7 PGPR strains, 1 local isolate of Phosphate Solubilizing Bacteria (PSB), and 2 control treatments: fertilized uninoculated (FUI) and unfertilized uninoculated (UFUI), with three replicates for each. Researchers treated chickpea seeds (var. JG-36), with various PGPR strains. The inorganic fertilizers were applied in three splits, through urea and a basal dose of SSP and MOP, according to the recommended dose of 20:80:20 kg ha^{-1} (N: P_2O_5: K_2O) for chickpea. The 75% of RDF through fertilizers were applied in inoculated plots only.

Table 1: Treatment details

Treatment	Details
T_1	(*Enterobacter asburiae* strain R31)
T_2	(*Microbacterium paraoxydans* strain IUURM B5)
T_3	(*Bacillus megaterium* strain BS5)
T_4	(*Bacillus megaterium* strain 10A-BS5)
T_5	(*Bacillus subtilis* strain F11)
T_6	(*Bacillus* sp. strain WM13-24)
T_7	(*Bacterium* 8-gw2 10)
T_8	(PSB: local isolate)
T_9	(FUI)
T_{10}	(UFUI)

Soil enzymatic activity

Acid and alkaline phosphatase activity

Phosphomonoesterases, crucial for mineralizing soil organic phosphorus, catalyze the hydrolysis of phosphoric acid esters and anhydrides. These enzymes are classified as acidic or alkaline based on their activity at different pH levels. The Tabatabai and Bremner (1969) method is used to measure phosphatase activity, employing colorimetric estimates of p-nitrophenol released from soil incubated with Modified Universal Buffer (MUB) at pH 6.5 for acid phosphatase and pH 11 for alkaline phosphatase.

Dehydrogenase activity

According to Burns (1978), dehydrogenase activity can be assessed by measuring the rate at which 2, 3, 5-triphenyl tetrazolium chloride (TTC) converts to triphenyl formazan (TPF). The formation of triphenyl formazan (TPF) correlates with biological activity, where higher biological activity results in a faster development of TPF.

Urease activity

The Urease activity was estimated by Bremner and Douglas (1971), in this method the urea hydrolysis in soils by determining the remaining urea after incubating the soil with a solution at 37°C. Urease activity is measured by calculating the difference between the amount of urea initially added and the amount recovered after a specified incubation period.

Microbial population

Rhizospheric soil samples were collected as fresh as possible and stored without grinding, sieving, or any modification, in order to preserve microbial integrity.The samples were placed in low-density polythene bags and refrigerated at 4°C until further analysis, minimizing changes in microbial populations.

Rhizobium and PSB population in soil

Soil was periodically collected and suspended in 90/mL of sterile water by adding 10/g of soil to flasks, which were then thoroughly shaken to produce a 10^{-2} dilution. Ten-fold serial dilutions were subsequently performed up to 10^{-9} for plating aliquots from each dilution were placed on selective media, and colony-forming units (CFUs) of *Rhizobium* and PSB were enumerated by David and Davidson, 2014, the YEM media and Pikovskaya media growth medium were used for *Rhizobium* and PSB respectively.

Statistical analysis

Using a randomized block design (RBD), replication-wise tabular data for each parameter were statistically analyzed following the methodology of Chandel (2002) to determine significant differences among treatment means. For microbial population data with high variability, logarithmic transformation was applied before conducting an ANOVA.

Results and Discussion

Acid phosphatase activities in soil

Acid phosphatase is an enzyme that catalyzes the hydrolysis of organic phosphorus compounds into inorganic phosphate, which is readily available for plant uptake. After the harvest of chickpea, the presence of active acid phosphatase in the soil ensures the continued mineralization of organic phosphorus, which is essential for the phosphorus needs of subsequent crops (Tarafdar & Claassen 1988). PGPR strains are being tabulated in Table 3. Acid phosphatase activities range from 13.46 to 20.61 µg PNP h^{-1} g^{-1} soil, with an average of 16.23 µg PNP $h^{-1}g^{-1}$ of soil.

The maximum phosphatase activities of 20.61 µg PNP h^{-1} g^{-1} oil was found T_1 (*Enterobacter asburiae* strain R31), which was higher than the FUI control. This might be due to the phosphate-solubilizing capabilities of *Enterobacter asburiae*. It can convert insoluble forms of phosphorus (e.g., rock phosphate) into soluble forms that are easily taken up by plants. This conversion process often involves the secretion of organic acids and phosphatases, including acid phosphatase, which catalyzes the hydrolysis of organic phosphorus compounds into inorganic phosphate (Rodríguez & Fraga, 1999). The inoculation with *Enterobacter asburiae* likely led to an increase in microbial biomass and diversity in the rhizosphere. Higher microbial activity often results in increased production of soil enzymes, including phosphatases. The 42% higher phosphatase activity in the T_1 treatment compared to the FUI control (14.55 µg PNP h^{-1} g^{-1} soil) suggests that *Enterobacter asburiae* significantly stimulated microbial processes that contribute to phosphorus mineralization (Saharan & Nehra, 2011).

The treatments T_7 and T_8 showed activities of 18.12 and 17.39 µg PNP h^{-1} g^{-1} soil, respectively with percent responses of 25 and 20 % from the control FUI follow the similar trends.

Alkaline phosphatase activities in soil

Alkaline phosphatase is an enzyme that catalyzes the hydrolysis of organic phosphorus compounds (such as phytates and phosphomonoesters) into inorganic phosphate (Pi) (McLaren & Cameron, 1996). The inorganic phosphate released by the activity of alkaline phosphatase becomes available for uptake by plants and microorganisms. This is crucial for plant nutrition and supports subsequent plant growth (Nannipieri, *et al*, 1990). The highest alkaline phosphatase activities (29.30 µg PNP h^{-1} g^{-1} oil) was monitored by inoculation of *Enterobacter asburiae* strain R31 (T_1), while the lowest was recorded in the UFUI.

The highest alkaline phosphatase activities of 29.30 µg PNP $h^{-1}g^{-1}$ soil was exhibited by *Enterobacter asburiae* strain R31 (T_1) which represented a 28% increase compared to FUI control. *Enterobacter asburiae* can enhance phosphorus availability by producing alkaline phosphatase enzymes. Alkaline phosphatase hydrolyzes organic phosphates into inorganic forms that plants can absorb (Richardson *et al.,* 2009). Next superior treatments are T_7 and T_8 which showed increments of 18 and 15%, respectively over the FUI control. Phosphatases are enzymes that release phosphate ions from organic compounds, making phosphorus available for plant uptake. PGPR strains, particularly phosphate-solubilizing bacteria, can significantly increase soil phosphatase activity, thereby enhancing phosphorus availability. In chickpea, inoculation with PGPR has been reported to increase acid and alkaline phosphatase activities, as shown in a study by (Gupta *et al.* 2012). PGPRs can alter the soil microbial community, leading to an increase in the population of microbes involved in enzyme production. This change can lead to enhanced enzyme activity, including alkaline phosphatase (Sinha and Ghosh, 2010).

Dehydrogenase activities (DHA) in soil

In the soil, DHA activity reflects the rate at which microorganisms are metabolizing organic materials. Since dehydrogenase enzymes are active only within viable, living cells, their activity is a direct reflection of microbial vitality and soil health. High DHA levels indicate an active and healthy microbial community capable of supporting robust soil processes (Alef & Nannipieri, 1995).The result indicated that, dehydrogenase activities in the rhizospheric soils of chickpea due to the inoculation of microbial strains, varied from 2.77 to 6.56 µg TPF h^{-1} g^{-1} soil, with an average activity of 4.49 µg TPF h^{-1} g^{-1} soil (Table 3).

The highest dehydrogenase activities of 6.56 µg TPF h^{-1} g^{-1} was received in T_1 (*Enterobacter asburiae* strain R31) which was 62% higher than the fertilized uninoculated control. It might be due to the ability of *Enterobacter asburiae* to produce phytohormones, siderophores, and other secondary metabolites, thereby stimulating microbial communities in the rhizosphere and leading to enhanced dehydrogenase activity, is supported by several studies (Asghar *et al.,* 2002)**.**This was followed by (T_7) and (T_8), with dehydrogenase activities which showed the increments of 35 and 25% respectively, compare to the FUI control.

Dehydrogenase is an important indicator of microbial oxidative activity in soil. PGPR strains have been shown to enhance dehydrogenase activity, indicating increased microbial activity and improved soil health. Singh *et al.* (2014) demonstrated that chickpea rhizosphere inoculated with *Bacillus* strains exhibited higher dehydrogenase activity compared to uninoculated controls.

Urease activities in soil

Urease is an enzyme that plays a crucial role in the nitrogen cycle, particularly in the breakdown of urea into ammonia and carbon dioxide. This process is significant in agricultural soils, especially after the harvest of leguminous crops like chickpea, which are known for their nitrogen-fixing abilities (Mobley &Hausinger, 1989). The data pertaining to urease activities in the rhizospheric soils of chickpea due to the inoculation of microbial strains found in between 2.67 to 7.07 µg urea hydrolyzed h^{-1} g^{-1} soil, with an average activity of 4.73 µg urea hydrolyzed h^{-1} g^{-1} in soil.

The maximum urease activity of 7.07 µg TPFµg urea hydrolyzed h^{-1} g^{-1} was reported in T_1 which was 96 % higher than the fertilized uninoculated control. *Enterobacter* spp. can promote root exudation by producing phytohormones like indole-3-acetic acid (IAA). These exudates, in turn, stimulate microbial communities, leading to increased microbial activity, including urease production. The presence of *Enterobacter* in the rhizosphere can thus lead to higher urease activity as the microbial population responds to the richer environment created by root exudates (Bhattacharyya and Jha, 2012). Some strains of *Enterobacter* possess the genetic capability to produce urease directly. This enzyme catalyzes the hydrolysis of urea into ammonia and carbon dioxide. When *Enterobacter* spp. are introduced into the soil, they can increase the overall urease concentration by producing it themselves, thereby boosting urease activity in the rhizosphere (Mobley *et al.,* 1995).

This was followed by T_7, T_8, T_3 and T_5 along with urease activities of 6.07, 5.38, 5.21 and 5.02 µg urea hydrolyzed $h^{-1}g^{-1}$, respectively, by responses of 68, 49, 44 and 39% over the control (FUI). Urease catalyzes the hydrolysis of urea into ammonia and carbon dioxide, a critical step in nitrogen cycling. PGPR strains, such as those producing urease or enhancing its activity, can improve nitrogen availability in the soil. A study by Arora *et al.* (2011) showed that PGPR inoculation in chickpea fields increased urease activity, contributing to better nitrogen cycling and utilization in the soil.

Table 3 : Impact of PGPR on the enzymatic activities in surface soil after harvest of chickpea

Treatment	**Phosphatase** (µg PNP $hr^{-1}g^{-1}$soil)		**DHA** (ugTPF$g^{-1}hr^{-1}$ soil)	**Urease activities** (µg urea hydrolyzed $g^{-1}hr^{-1)}$ Soil)
	Acid	Alkaline		
T_1 (*Enterobacter asburiae* strain R31)	20.61	29.3	6.56	7.07
T_2 (*Microbacterium paraoxydans* strain IUURM B5)	15.41	23.15	3.73	3.66
T_3 (*Bacillus megaterium* strain BS5	16.34	25.43	4.17	5.21
T_4 (*Bacillus megaterium* strain 10A-BS5)	15.32	22.47	3.98	4.15
T_5 (*Bacillus subtilis* strain F11)	16.17	25.47	4.33	5.02
T_6 (*Bacillus* sp. strain WM13-24)	13.86	24.75	4.87	4.49
T_7 (*Bacterium* 8-gw2-10)	18.12	27.02	5.45	6.07
T_8 (PSB: local isolate)	17.39	26.28	5.06	5.38
T_9 (FUI)	14.55	22.79	4.03	3.6
T_{10} (UFUI)	13.46	21.38	2.77	2.67
Mean	16.23	24.80	4.49	4.73
SEm ±	0.88	1.13	0.32	0.47
CD (P=0.05)	2.48	3.2	0.9	1.34

Microbial population in chickpea rhizosphere

The *Rhizobium* population in surface soil at the harvest of chickpea was significantly impacted by various PGPR strains and illustrated in Table 4. The *Rhizobium* counts ranged from 6.23 log cfu (16.98 × 10^5cfu g^{-1} soil) to 7.80 log cfu (63.09 ×10^6cfu g^{-1} soil), with a mean value of 7.26 log cfu (18.32 × 10^6cfu g^{-1} soil). Most of the PGPR strains were shown significant synergistic effects with *Rhizobium* rhizospheric soil at post-harvest of chickpea, except for treatments T_2 and T_6, compared to FUI.

The maximum *Rhizobium* sp. count of 7.80 log cfu (63.09 ×10^6cfu g^{-1} soil), was observed through inoculation of *Enterobacter asburiae* strain R31 (T_1), than that of control FUI. *Enterobacter* spp. can enhance the overall microbial community structure in the rhizosphere, providing a more favorable environment for *Rhizobium* spp. through synergistic interactions. This improved rhizosphere environment can lead to increased colonization and growth of *Rhizobium* spp., as they benefit from the altered microbial dynamics and enhanced nutrient availability (Glick, 1999 and 2012). Some *Enterobacter* strains are known to induce systemic resistance in plants, which can also create a more favorable environment for beneficial microbes like *Rhizobium* spp. by reducing plant stress and enhancing overall plant health (Van Loon, 2007). It was followed by T_7 (*Bacterium* 8-gw2-10) of 7.72 log cfu (52.48× 10^6cfu g^{-1} soil), T_8 (PSB: local isolate) of 7.66 log cfu (52.48× 10^6cfu g^{-1}soil), T_5 (*Bacillus subtilis* strain F11) of 7.65 log cfu(44.66× 10^6cfu g^{-1} soil),T_4 (*Bacillus megaterium* strain 10A-BS5) of 7.45 log cfu(28.18× 10^6cfu g^{-1} soil) and T_3 (*Bacillus megaterium* strain BS5) of 7.38 log cfu (23.98× 10^6cfu g^{-1} soil).

Population of PSB

PGPR can stimulate root growth and increase the exudation of organic compounds from the roots. This can create a more favorable environment for PSB if they are adapted to utilize these exudates, or it can inhibit them if the changes create less favorable conditions (Badri *et al.,* 2009). Results revealed that the inoculation of PGPR strains significantly influenced the population of PSB in chickpea rhizospheric soil (Table 4). The population increased from 7.06 log cfu (11.48 × 10^6cfu g^{-1}soil) to 8.83 log cfu (67.60 × 10^7cfu g^{-1} soil) with an average value of 8.19 log cfu (15.48 × 10^7cfu g^{-1} soil). Most of the PGPR strains reported significant performances in the PSB population count in the rhizospheric soil of chickpea.

The inoculation of T_1 had exhibited significantly maximum population count of PSB sp. with a value of 8.83 log cfu (67.60 × 10^7cfu g^{-1} soil) as compared to control FUI. *Enterobacterasburiae*R31 might possess the ability to solubilize phosphate more efficiently than other strains. Phosphate solubilisation is a critical trait for PSB, as it makes phosphorus more available to plants and can thus promote the proliferation of these bacteria. This increased availability of phosphorus can support the growth of the inoculated PSB, leading to a higher population count (Rodriguez and Fraga, 1999). The interaction between *Enterobacterasburiae*R31 and the existing microbial community in the soil could be synergistic. This means that the presence of R31 might create a more favorable environment for the growth of PSB through various interactions, such as improved soil structure or altered microbial interactions (Glick, 2012).

This was followed by T_7 (*Bacterium* 8-gw2-10), of 8.80log cfu (63.09× 10^7cfu g^{-1} soil),T_8 (PSB: local Isolate) of 8.78 logcfu(60.25× 10^7cfu g^{-1} soil), T_5 (*Bacillus subtilis* strain F11) of 8.65 logcfu(44.66× 10^7cfu g^{-1} soil), T_6 (*Bacillus* sp. strain WM13-24) of 8.54 logcfu(34.67× 10^7cfu g^{-1} soil) and T_4 (*Bacillus megaterium* strain 10A-BS5) of 8.51 logcfu (32.35× 10^7cfu g^{-1} soil). Different bacterial strains possess unique characteristics that influence their ability to survive, proliferate, and interact with their environment. For instance, *Bacillus subtilis* and *Bacillus megaterium* are known for their robust growth and ability to produce various enzymes and metabolites that can affect their efficacy in phosphate solubilization and overall microbial activity (Khan *et al.,* 2009).

Table 4: Impact of PGPR on the microbial population in Rhizospheric soil of chickpea

Treatments	**Microbial population (log cfu)**	
	Rhizobium	**PSB**
T_1 (*Enterobacter asburiae* strain R31)	7.80(63.09 X 10^6)	8.83(67.60 X 10^7)
T_2 (*Microbacterium paraoxydans* strain IUURM B5)	6.97(93.32X 10^5)	7.67(57.54 X 10^6)
T_3 (*Bacillus megaterium* strain BS5)	7.38(23.98 X 10^6)	7.62(41.68 X 10^6)
T_4 (*Bacillus megaterium* strain 10A-BS5)	7.45(28.18X 10^6)	8.51(32.35 X 10^7)
T_5 (*Bacillus subtilis* strain F11)	7.65(44.66 X 10^6)	8.64(44.66X 10^7)
T_6 (*Bacillus* sp. strain WM13-24)	6.99(9.77 X 10^5)	8.54(34.67 X 10^7)
T_7 (*Bacterium* 8-gw2-10)	7.72(52.48 X 10^6)	8.80(63.09 X 10^7)
T_8(PSB: local isolate)	7.66(45.70 X 10^6)	8.78(60.25 X 10^7)
T_9 (FUI)	6.78(60.25 X 10^5)	7.52(33.11 X 10^6)
T_{10} (UFUI)	6.23(16.98 X 10^5)	7.06(11.48 X 10^6)
Mean	7.26 (18.32 × 10^6)	8.19 (15.48 × 10^7)
SEm ±	0.15	0.17
CD (P=0.05)	0.43	0.47

Conclusion

Overall, the study highlight significant benefits of application chickpea with the PGPR strain *Enterobacter asburiae* R31 (T1). This strain best performance others in improving Acid phosphatase activity with increase 42%, Alkaline phosphatase activity 28%, Dehydrogenase activity 62 %, Urease activity 96%, *Rhizobium* population of 7.80 log cfu (63.09×10^6cfu g^{-1} soil) and Population of PSB of 8.83 log cfu (67.60×10^7cfu g^{-1} soil)compared to the fertilized uninoculated control (FUI). Other strains, such as T_7 (*Bacterium* 8-gw2 10) T_8 (PSB: local isolate) T_5 (*Bacillus subtilis* strain F11) T_3 *(Bacillus megaterium* BS5) and T_4 (*Bacillus megaterium* 10A BS5), also showed positive results in improving soil properties and microbial population.

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