

**ISOLATION, IDENTIFICATION AND EVALUATION OF
PHOSPHATE AND ZINC SOLUBILIZERS IN WHEAT
RHIZOSPHERE**

h/t

by
Ravi Kumar
(J-20-M-762)

**A Thesis submitted to
Faculty of Agriculture
in partial fulfillment of the requirements
for the degree of**

**MASTER OF SCIENCE IN AGRICULTURE
SOIL SCIENCE AND AGRICULTURAL CHEMISTRY**



Division of Soil Science and Agricultural Chemistry
Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu,
Main Campus, Chatha, Jammu – 180009
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CERTIFICATE - I

This is to certify that the thesis entitled, "Isolation, Identification and Evaluation of phosphate and zinc solubilizers in wheat rhizosphere" submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture (Soil Science and Agricultural Chemistry)** to the Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, is original work and has similarities with published work not more than minor similarities as per UGC norms of 2018 adopted by the University. Further, the level of minor similarities has been declared after checking the manuscript with **URKUND** software provided by the University.

The work has been carried out by **Mr. Ravi Kumar**, Registration No. **J-20-M-762** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.



Dr. Renu Gupta

(Major Advisor)

Place: Jammu

Date: 25/8/2022



Head of the Division



Dean FoA

CERTIFICATE- II

We, the members of the advisory Committee of **Mr. Ravi Kumar**, Registration No. **J-20-M-762**, a candidate of the degree of **Master of Science in Agriculture (Soil Science and Agricultural Chemistry)** have gone through the manuscript of the thesis entitled, "Isolation, Identification and Evaluation of phosphate and zinc solubilizers in wheat rhizosphere" and recommend that it may be submitted by the student in partial fulfilment of the requirements for the degree.



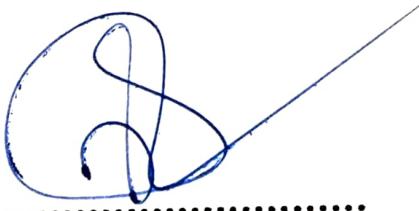
Dr. Renu Gupta
Associate Professor
Major Advisor &
Chairman Advisory Committee

Place: Jammu

Date: 25/8/2022

Advisory Committee Members

Dr. Vikas Sharma
Professor & Head
Division of Soil Science and
Agricultural Chemistry
(Member from Major Subject)


.....

Dr. Susheel Sharma
Assistant Professor
School of Biotechnology
(Member from Minor Subject)


.....

Dr. Rakesh Nanda
Professor & Head
Division of Agricultural Extension Education
(Dean's Nominee)


.....

CERTIFICATE-III

This is to certify that the thesis entitled "**Isolation, Identification and Evaluation of phosphate and zinc solubilizers in wheat rhizosphere**", submitted by **Mr. Ravi Kumar**, Registration No. **J-20-M-762**, to the Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture (Soil Science and Agricultural Chemistry)**, was examined and approved by the advisory committee and external examiner(s) on **07-10-2022**.

Naveen Dutt
07/10/22

External Examiner

Dr. Naveen Dutt

Principal Scientist

Department of Soil Science

CSKHPKV Palampur

Renu
7/10/22

Dr. Renu Gupta
Associate Professor
Soil Science and Agricultural Chemistry
(Major Advisor)

D
Head
Division of Soil Science and Agricultural Chemistry

D
Dean

Faculty of Agriculture
SKUAST-Jammu

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Ravi Kumar

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ABSTRACT

Title of Thesis	:	“Isolation, Identification and Evaluation of phosphate and zinc solubilizers in wheat rhizosphere”
Name of student	:	Ravi Kumar
Registration No.	:	J-20-M-762
Major Subject	:	Soil Science and Agricultural Chemistry
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ABSTRACT

The present study entitled “Isolation, Identification and Evaluation of phosphate and zinc Solubilizers in wheat rhizosphere” was carried out at Division of Soil Science and Agricultural Chemistry of Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu during 2021-2022. An attempt was made for identification and evaluation of PSRB & ZnSRB isolates from the wheat rhizosphere in Jammu region (J&K- UT). *In vitro* experiment was carried out to isolate and characterize the PSRB & ZnSRB isolates for various PGPR activities. Biochemical characterization was performed for isolates viz. Citrate utilization, Urease, Nitrate reduction, and H₂S production and based on these biochemical tests, these were subjected to identification. Qualitative estimation of phosphate solubilization activity of PZn and ZnP isolates in PVK agar plates revealed high phosphate solubilization index of (0.62) and (0.50) respectively at 9th day with PZn-2 isolate. Quantitative estimation of phosphate solubilization of PZn and ZnP isolates in PVK broth and ZnS broth was recorded highest in PZn-2 isolate with 0.651 µg/ml at 420 nm wavelength at 13th day interval. Qualitative estimation of zinc solubilization activity of ZnP and PZn isolates in ZnS agar culture plates revealed SI of ZnP-1 isolate and was found to be highest 0.35 and 0.55 at 7th and 9th day respectively among all the isolates. Maximum Zn solubilization was observed at 9th day. Quantitative estimation of zinc solubilization of PZn and ZnP isolates in PVK broth and ZnS broth was recorded highest in ZnP-1 isolate viz., 9.50 µg/ml & 42.25 µg/ml at 9th and 13th day respectively. The study reveals that bacterial isolates exhibited maximum solubilization activity between 9th to 13th day for phosphate solubilization and zinc solubilization respectively.

Pot trial was laid out in factorial CRD with 4 replications and fifteen treatment combinations. The treatments consisted of P₀(control), P₁(TCP), P₂(ZnCO₃) and Broth treatment viz., B₀(control), B₁(PZn-1 isolate) B₂(PZn-2 isolate) B₃(ZnP-1 isolate) and B₄(consortium). Vigorous wheat seeds of variety HD-2967 were sterilized and then subjected to broth treatment prior to sowing in respective pots. Application of TCP, ZnCO₃ and consortium treatments significantly increased available nutrients in rhizospheric soil of wheat crop. Non-significant decrease in pH was recorded in all stages of wheat growth. Highest available N (91.43 mg/kg) at tillering stage, (92.74 mg/kg) at earhead initiation stage, available P (17.71 mg/kg) at tillering stage, (19.35 mg/kg) at earhead initiation stage, available K (91.48 mg/kg) at tillering stage, (93.66 mg/kg) at earhead initiation stage and available Zn (1.63 mg/kg) at tillering stage, (1.70 mg/kg) at earhead initiation stage were recorded with treatment P₁ & Consortium. P₁ amended P-Zn isolates consortium showed significant increase in SMBC and dehydrogenase activity. Highest SMBC (92.53 µg⁻¹ soil) at tillering stage and (93.97 µg⁻¹ soil) at earhead initiation stage and highest dehydrogenase activity (57.82 µg-TPF g⁻¹soil hr⁻¹) at tillering stage, (59.10 µg-TPF g⁻¹soil hr⁻¹) at earhead initiation stage were recorded with treatment P₁. Maximum grain yield with (18.56 g/pot) and N uptake (20.11 g/pot), P uptake (4.73 g/pot), K uptake (4.87 g/pot) and Zn uptake (27.25 g/pot) was recorded with treatment P₁, P₂ & B₄ (consortium) in wheat crop. Based on present experimental results from this investigation it was concluded that among the identified isolates, PZn-2 isolate proved to be an efficient strain in phosphate solubilization whereas ZnP-1 isolate was more efficient among all in zinc solubilization in wheat crop. Further, soil amended with TCP, ZnCO₃ along with consortium and sole use of single isolate resulted in better proliferation of soil microbes, improved rhizosphere soil and better yield in wheat crop.

Keywords: Wheat, rhizosphere, phosphate solubilization, zinc solubilization, enzyme activity



Signature of Major Advisor



Signature of Student

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ABBREVIATIONS

@	=	At the rate of
PVK	=	Pikovskaya
ZnS	=	Zinc Solubilizing
°C	=	Degree Celsius
%	=	Percent
Fig	=	Figure
mg	=	Milligram
kg ⁻¹	=	Per Kilogram
Av.	=	Available
N	=	Nitrogen
P	=	Phosphorus
K	=	Potassium
Zn	=	Zinc
Mg	=	Magnesium
Ca	=	Calcium
h ⁻¹	=	Per Hour
soil ⁻¹	=	Per soil
ZnSRB	=	Zinc Solubilizing Rhizobacteria
PSRB	=	Phosphate Solubilizing Rhizobacteria
TCP	=	Tri Calcium Phosphate
ZnCO ₃	=	Zinc Carbonate
g ⁻¹	=	Per gram
P-Zn	=	Phosphorus-Zinc
<i>et al</i>	=	And others
SMBC	=	Soil Microbial Biomass Carbon
PGPR	=	Plant growth promoting rhizobacteria
ZnO	=	Zinc Oxide

Introduction

Chapter 1

Introduction

Wheat (*Triticum aestivum* L.) is one of the three major cereals (along with maize and rice), a major source of energy, renewable resource for food, feed, industrial raw material, source of protein (8-15%), fibre in the human diet and a staple food crop for more than one-third of the world's population. Wheat is considered as "The King of Cereals" because it can be grown in a wider range of soils and temperatures than any other grain and it is better suited for bread manufacturing and used as a staple food. Overall world production volume of wheat is 772 million metric tonnes in the year 2020 (Anonymous, 2021). In India, wheat is second most important food crop, next only to rice, which is about 13% share of world's wheat production and ranks second in production next to China which has about 17% share of world's wheat production. According to Directorate of Economics and Statistics DAC&FW in year 2019-20 estimates, the area under wheat cultivation all over India is 31.45 million hectares whereas, production & yield are 107.59 million tonnes and 3421 kg ha⁻¹ respectively. Uttar Pradesh is leading with area under cultivation of 9.50 million hectares and percentage share of area all over India is 30.19% with 32.59 million tonnes of production and 3432 kg ha⁻¹ of yield. In J&K UT, the total area under cultivation is 286.02 thousand hectares, out of which 282.5 thousand hectares area is being cultivated in Jammu division with considerably high average yield (Anonymous, 2020). It is grown both as a spring and winter crop.

Next to nitrogen, phosphorus (P) is the second most important macronutrient for increased plant development and yield. It is responsible for supplying, transferring and storing energy for all biochemical processes within the plant (Khan *et al.* 2009). Despite the high demand for enhanced crop output, just 0.1 percent of total soil P is soluble and available to plants. This is owing to phosphate fixation and limited solubility in soil (Pereira and Castro 2014). As a result, only soluble ionic phosphate (Pi) is efficient as a mineral nutrition. Sources of plant P in the form of P fertilizers such as TCP, SSP, rock phosphate when combined with two or more strains of PSRB are found to increase the efficiency of P resources by optimizing the chemical fertilizer application for crop production and making their use to replenish, maintain soil fertility and environment

safe as well. TCP is a mineral apatite and is found to be an easily degradable substrate than SSP. Thus its use along with PSRB is found to improve phosphate solubilization by releasing organic acids making the native and added P soluble which in-turn influence plant growth (Murugan *et al.*, 2013; Gulati *et al.*, 2010; Kshetri *et al.*, 2018).

Microbial-mediated phosphorus management is gaining popularity as an environmentally benign and cost-effective method of soil phosphorus nourishment. Soil microbes enhance plant nutrient accumulation. They are involved in a broad range of biological processes including the transformation of unavailable and insoluble soil nutrients (Babalola and Glick 2012). The ability of phosphate solubilizing rhizobacteria (PSRB) to convert insoluble forms of phosphorous into forms that are accessible to plants, such as orthophosphate, is a key characteristic in improving crop development and output. The ability of phosphate solubilizing bacteria to convert insoluble P to soluble P through the release of organic acids, chelation and ion exchange promotes soil fertility exchange (Omar 1997; Narula *et al.* 2000; Whitelaw 1999). The main active strains in this conversion belong to a range of genera, including *Pseudomonas*, *Mycobacterium*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Gordonia*, *Phyllobacterium*, *Delftia*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Micrococcus*, *Bacillus*, *Flavobacterium*, *Rhizobium*, *Mesorhizobium* and *Sinorhizobium* (Chen *et al.* 2006; Chung *et al.* 2005).

In India, up to 50% of the agricultural land, particularly the whole of the Indo-Gangetic belt, is reeling under zinc deficiency. This has serious repercussions as plants grown on zinc-deficient soils have decreased grain yield (~80%). The major reason for widespread occurrence of zinc deficiency problem in crop plants is especially its low solubility rather than a total low amount of zinc (Cakmak, 2008). Likewise, Zinc (Zn) is a necessary trace element for plant growth and reproduction and plays an important part in many biological processes. Zinc (Zn) is essential for agricultural production as well as human growth. Because Zn is a regulatory co-factor and structural constituent in proteins and enzymes involved in many metabolic pathways, a shortage can have major consequences for plant and human development (Alloway 2009; Cakmak *et al.* 2017). Despite the fact that zinc is only required in trace amounts, zinc deficiency is common in wheat during the crop growth phases and its availability is influenced by a variety of factors including soil pH, soil texture, soil phosphorus, soil type and weather conditions (Alloway 2009). Zn deficiency in agricultural soils is the most widely

distributed micronutrient deficiency constraining crop productivity (yield losses can exceed 40%), whereas excess Zn in soil can be either geological or anthropogenic in origin. Less Zn availability (high pH problematic soils), less total soil Zn, loss of soil organic matter, absence of N, Na, Ca, Mg and PO₄, restricted root exploration and climate factors are the most common causes of Zn deficiency in crops. Its deficiency is displayed as stunted growth, reduced inter-nodal length, young leaves are smaller than normal. Zinc deficiency results in reduced membrane integrity, reduction in synthesis of carbohydrates, cytochromes, nucleotides, auxins, chlorophyll and increased susceptibility for heat stress. Root cell membrane permeability is increased under zinc deficiency which might be related to the function of zinc in cell membranes (Parker *et al.* 1992; Singh *et al.* 2021). One of the widest ranging abiotic stresses in the world agriculture arises from the low zinc (Zn) availability in the calcareous soils. Availability of Zn to plant is hindered by its immobile nature and unfavourable soil conditions. The solubility of zinc is highly dependent on soil pH and moisture and therefore arid and semi-arid areas are often zinc deficient. It can be corrected through exogenous application of soluble zinc sources like ZnCO₃, ZnO, ZnSO₄ etc. but only 20% of applied zinc is available for plant uptake and rest of the zinc is converted to various unavailable forms. Zinc thus made unavailable is converted back to available form accessible to plants, such as divalent zinc cation, by inoculating bacterial strains which can solubilize it by release of organic acids and decline in pH (Khatoon *et al.* 2022). It is a key feature in improving crop plant development and output. Zinc solubilizing bacteria are viable zinc supplementation alternatives because they transform applied inorganic zinc to usable forms. Zinc deficiency has a significant impact on wheat yield and local studies have indicated that zinc fertilizers can help in increasing Zn availability to crop plants (Khan *et al.* 2009; Ahmad *et al.* 2012; Joy *et al.* 2017).

Microbes in the rhizosphere have long been recognized as direct plant growth enhancers. Soil microorganisms adapt to changes in soil conditions and play a critical role in maintenance of soil fertility. Microbial activity is linked to the mineralization of essential nutrients that play an important part in the biogeochemical cycle of C, N, P and other nutrients, which are otherwise unavailable to plants in complex forms. Since wheat belt of Jammu region is nitrogen, phosphorus and zinc deficient, to cope up with this deficiency and to improve the nutrient status of soil, farmers usually apply inorganic fertilizers which have deteriorated the soil health in terms of low nutrient

availability. The use of these inoculants can improve the productivity of commonly and artificially created P and Zn resources and can augment the use of chemical fertilizer for crop production (Salimpour *et al.* 2010) and can be studied further for identification using existing data.

PSRB and ZnSRB isolates from indigenous wheat crops could be used as an alternate technique to ameliorate soluble phosphorus, zinc and macronutrient deficiencies in the wheat rhizosphere as PSRB and ZnSRB isolates contain a wide range of unrelated bacteria (Khan *et al.* 2006).

Therefore, the present study entitled "**Isolation, Identification and Evaluation of phosphate and zinc Solubilizers in wheat rhizosphere**" was undertaken with the following objectives:

- Isolation and identification of phosphate and zinc solubilizers from wheat rhizosphere
- To study effect of PSRB and ZnSRB isolates on rhizospheric properties and yield attributes in wheat

Review of Literature

Chapter 2

Review of Literature

Phosphorus and zinc plays an important role in plant growth, development and must be made available to plants for better results. Despite the fact that both organic and inorganic phosphorus are abundant in soils, it is typically a major limiting factor for plant growth. Likewise, zinc deficiency is widespread in dry climatic condition mainly owed to low zinc solubility and high fixation. Due to the nutrient fixation characteristics of applied phosphatic and zinc fertilizers, as well as the negative effects of use of chemical fertilizer on soil microbial flora, rhizospheric properties and crop yield, it is necessary to use phosphate and zinc solubilizing rhizobacterial isolates from native crop rhizosphere to improve its efficiency in site-specific crops.

The literature pertaining to the present study has been reviewed and presented below under appropriate headings which will provide an overview of current status of research work.

- 2.1 Isolation of phosphate and zinc solubilizers from rhizosphere**
- 2.2 Identification of Isolates**
- 2.3 Mechanisms of phosphate and zinc solubilization of PSRB and ZnSB isolates in culture medium**
- 2.4 Screening of isolates for solubilizing activity using various P and Zn sources**
- 2.5 Effect of PSRB and ZnSRB isolates on**
 - ✓ **Soil nutrients**
 - ✓ **Soil enzymes activity**
 - ✓ **Nutrient uptake**
 - ✓ **Yield attributes**

2.1 Isolation of phosphate and zinc solubilizers from rhizosphere

Silva and Ferreira (2010) isolated three bacterial strains from sugarcane leaves and roots. The ability of these isolates to solubilize insoluble nutrients in Liquid Glucose Ivo (LGI) medium containing tri calcium phosphate and zinc oxide was next tested. The isolate SCB4789F-1 was the most effective in phosphorus solubilization,

with a halo zone of solubilization diameter of 0.75 cm on average. The zinc isolates SCB4789F-1 and SCB4789F-2 had the best solubility, with an average halo zone diameter of 1.34 and 1.29 cm, respectively.

Kaushal *et al.* (2011) identified 40 isolates from cauliflower rhizosphere samples grown in three H.P. districts (Kangra, Hamirpur and Bilaspur). Only five isolates (MK2, MK4, MK5, MK7 and MK9) demonstrated the maximum plant growth-promoting characteristics, such as P solubilization and N fixation. MK5 and MK7 demonstrated the highest phosphate solubilizing efficiency among the five isolates, with 177.21 and 166.67 per cent, respectively. The 16S rRNA sequence analysis of MK5 and MK7 strains using the clustal W programme revealed that isolate MK5 had (99%) homology with *B. subtilis* (HQ594544) and isolate MK7 had (100%) homology with *B. pumilus* (GU290547), distinguishing the bacterial isolates on a genetic basis as both isolates clustered with *Bacillus sp.*

Kumar *et al.* (2011) reported that a total of 80 rhizobacteria was isolated from coastal agricultural ecosystem of cultivated vegetable rhizosphere soils and revealed that environmental *Staphylococcus* isolates were able to solubilize phosphate including calcium phosphate.

Xinxian *et al.* (2011) studied that zinc-solubilizing bacteria from the roots, stems and leaves of *Sedum alfredii*, a Zn/Cd hyper accumulator plant. A total of fourteen bacterial endophytes were recovered and 16S rRNA sequence analysis revealed that they are closely linked phylogenetically to *Pseudomonas*, *Bacillus*, *Stenotrophomonas* and *Acinetobacter*. Strains VI8L1, VI8L2, II8L4 and VI8R2 were able to successfully solubilize ZnCO₃ and Zn (PO₄)₂ in both plate and broth assays.

Sharma *et al.* (2012) isolated zinc solubilizing bacteria from soybean rhizosphere soils. On Tris-minimal agar medium supplied individually with 0.1 per cent zinc in the form of zinc oxide, zinc phosphate and zinc carbonate, these isolates were tested in vitro for zinc-solubilization capacity. Based on gram-positive reaction, endospore-forming cells and the presence of iso-C15:0 and anteiso-C15:0 as major fatty acids, 9 isolates were described and recognized as *Bacillus* spp. In liquid medium supplemented with zinc phosphate and zinc carbonate compounds, the isolates KHBD-6, KHBAR-1, BDSD-2-2C and KHTH-4-1 as well as the reference strain ATCC 13061, showed higher soluble zinc concentrations than the other isolates and uninoculated control.

Jha *et al.* (2013) discovered the mineral phosphate solubilization potential of bacterial strains isolated from the rhizosphere regions of three agriculturally important crop plants [viz., *Pennisetum glaucum* (cv. Raj 171), *Sesamum indicum* (cv. RT 46) and *Phaseolus aureus* (cv. RMG 492)], which were collected in the order DCP>TCP>FP from different locations on the Banasthali varsity campus.

Gusain *et al.* (2015) analyzed the 16S rRNA sequences of bacteria isolated from rain fed agriculture fields in the Garhwal Himalaya and discovered two bacterial strains, YB1 and YB3, with efficient P solubilization activity on solid medium. YB1 was identified as a *Pseudomonas koreensis* strain, YB3 as *Arthrobacter nitroguajacolicus* and YB2 as *Klebsiella oxytoca*, YB4,YB5 as two *Arthrobacter nitroguajacolicus* strains and YB1,YB2, YB3 strains also showed efficient phosphate solubilization activity at 28°C.

Linu *et al.* (2017) discovered that *Pseudomonas aeruginosa* PS-2 (KR270346) and *Pseudomonas aeruginosa* PS-3 (KR270347) were optimised under different growth conditions in culture medium including diverse carbon and nitrogen sources out of 12 bacterial strains isolated from chilli rhizosphere. At pH 7, temperature 30°C and with glucose and ammonium sulphate as the optimum carbon and nitrogen sources, both isolates demonstrated maximal P solubilization.

Mishra *et al.* (2017) conducted research to isolate and characterize zinc solubilizing bacteria (ZnSB) from the rice rhizosphere, as well as to evaluate these isolates for plant growth enhancement in rice seedlings. ZnSB satisfactorily solubilized both insoluble zinc compounds, however ZnO was more effectively solubilized than ZnCO₃. The soluble Zn concentration in the culture supernatant was determined using an atomic absorption spectrophotometer (AAS). Isolate Zn-3 had the maximum Zn solubilization in culture broth (62.48 mg/l). The majority of the isolates exhibited a link between Zn solubilization and a drop in pH in the culture medium. The nine ZnSB were found to be from four different genera in two phyla: proteobacteria and 13 proteobacteria.

Shakeela *et al.* (2017) reported that among 40 bacterial isolates isolated from the rhizosphere soil of the *Picrorhiza kurroa* , maximum P- solubilization was shown by five isolates (PkR (7a)*, Pk7(B), Pk14(b), Pk12(d) and PC-4) as 320.00 mg/l, 120.00 mg/l, 205.00 mg/l, 100.00 mg/l and 180.00 mg/l respectively in Pikovskaya's medium

and (PkR (7a)* was identified as *Bacillus subtilis* by 16S rRNA sequencing showed highest P- solubilization of 320.00 mg/l at pH 7.0, 0.5 per cent TCP concentration in PVK broth.

Santosa *et al.* (2018) isolated bacterial strains from the rhizosphere soil of IR 64 organic and inorganic rice fields in the district of Central Java, Indonesia. Nine of the ten colonies had morphological differences in the culture media and were isolated from an organic rice crop. Based on 16SrRNA sequence analysis nine isolates were identified as *Pseudomonas aeruginosa* strain (RI-98-1), *Stenotrophomonas maltophilia* strain (S431), *Bacillus subtilis* strain (CEB2), *Bacillus cereus* strain (ATCC 14579 clone EA195), *S. maltophilia* strain (5517), *Exiguobacterium acetylicum* strain (SSA-3), *Serratia nematodiphila* strain (HC4), *Bacillus cereus* strain (ANP221) and *Acinetobacter junii* strain (M. pstv. 21.4).

Dinesh *et al.* (2018) devised experiments to recover and identify the Zn solubilization capacity of a variety of bacterium isolates. Because of its improved Zn solubilization in vitro, ZnSB2 (*B. megaterium*, KY687496) was judged to be the most promising of the six promising Zn solubilizing bacteria (ZnSB).

Rai *et al.* (2019) isolated two bacterial strains *P. aeruginosa* and *P. fluorescens* from garden soil and checked for the potential of Phosphate and zinc solubilization successfully. During this study they also revealed that the isolate *P. fluorescens* was found more potent for PGPR activity.

Eramma *et al.* (2020) carried out a study for isolation and testing of PSB solubilization efficiency on Pikovskaya agar and broth at various incubation times. From the paddy rhizosphere soils of Raichur and Koppal districts, a total of 40 PSB were isolated. The zone of solubilization, solubilization efficiency, solubilization index, pH change titrable acidity and phosphatase activity of PSB isolates were all investigated. On the sixth day of the incubation period, the solubilization zone, efficiency and index were at their peak. Ten isolates were discovered to be effective phosphate solubilizers. On Pikovskaya medium, isolate PPSB-21 had the maximum zone of solubilization (18.4 mm), phosphate solubilization efficiency (253.84 per cent) and solubilization index (3.53) among the ten isolates. Biofertilizers can be made from these effective isolates.

Gupta *et al.* (2022) recovered 37 isolates from rice rhizosphere in Jammu district of J&K UT. The isolates were subjected to halo zone development for checking solubilizing activity and 12 isolates showed halo zone development.

2.2 Identification of Isolates.

Joshi and Bhatt (2011) discovered a sum of 133 isolates associated with wheat rhizosphere from northern Himalayas. *Bacillus sp.* (44 per cent) was the most prevalent genus, followed by *Pseudomonas sp.* (24 per cent), *Serratia sp.*, *Flavobacterium sp.* (7 per cent each), *Micrococcus sp.* (3 per cent), *Klebsiella sp.* (4 per cent), *Azotobacter sp.* (6 per cent), *Enterobacter sp.* (4 per cent), *Staphylococcus sp.* and *Micrococcus sp.* were frequently present. Following biochemical and morphological testing, all of the genera were provisionally identified.

Maheswar and Sathiyavani (2012) investigated the bacterial strains obtained from groundnut rhizosphere soil. The isolated colony was identified as *Bacillus subtilis* and *Bacillus cereus* after morphological and biochemical analysis. The isolates were optimised for phosphate solubilization at varied pH (5-9), temperature (25-45°C) and carbon, nitrogen and potash sources. At pH 7, *Bacillus subtilis* was shown to be the most efficient for phosphate solubilization (0.35 ± 0.02 mg/l), carbon sources (0.28 ± 0.03 mg/l), nitrogen sources (0.28 ± 0.03 mg/l) and potash sources (0.12 ± 0.09 mg/l).

Gupta *et al.* (2022) identified four distinct isolates using 16S primers as *Pseudomonas aeruginosa*, *Bacillus subtilis* strain 1, *B. subtilis* strain 2, and *B. subtilis* strain 3, respectively. Screening for phosphate solubilization activity revealed that the halo zone diameter formed by these isolates extended from 2.1 to 3.2 mm. The phosphate solubilizing efficiency (SE) ranged from 35.4 to 50.9, with PS4 recording the highest value of 50.9 and the highest P solubilization of 10.22 g/ml on the 7th day. The phosphorus solubilization potential of these identified PSRB strains can be used and investigated in the phosphorus-deficient rice growing areas of Jammu.

2.3 Mechanisms of phosphate and zinc solubilization of PSRB and ZnSB isolates in culture medium.

Saravanan *et al.* (2004) evaluated the zinc solubilizing potential capacity of *Bacillus sp.* and *Pseudomonas sp.* using zinc oxide, zinc sulphide (Sphalerite) and zinc carbonate in both plate and broth experiments. On the fifteenth day following inoculation, ZSB-O-1 (*Bacillus sp.*) displayed the highest dissolution in the zinc

sulphide (Sphalerite ore), with 2.80 cm of dissolution zone, 14.50 cm² of area in the plate assay and 13.60 mg kg⁻¹ of zinc in the broth assay. With a 3.30 cm clearing zone and 20.43 cm² area in the plate test and 16.40 mg kg⁻¹ of zinc in the broth assay throughout the same inoculation period, the ZSB-S-2 (*Pseudomonas sp.*) demonstrated greater solubilizing ability in the zinc oxide.

Chakkaravarthy *et al.* (2010) investigated the phosphate solubilization activity of the PB08 *Bacillus* strain in two media with two phosphorus sources (TCP and phosphate sludge), finding a gradual increase in phosphate solubilization activity with values of tri calcium phosphate solubilized from 9.6 to 133.6 ppm for TCP and 12.6 to 136 ppm for phosphate sludge and The pH dropped from 7.04 (control) to 3.0 (TCP) on the third day and 3.38 (PS) on the ninth day, resulting in a solubilization index of 4.08 by *Bacillus* strain.

Tripti *et al.* (2012), reported that the isolates S2 and S30, which were identified as *Bacillus* and *Pseudomonas sp.* had the highest phosphate solubilization index of 3.1 and 3.0 in PVK agar plates, as well as high soluble phosphate production of 373.07 mg l⁻¹ and 368.58 mg l⁻¹ in broth culture, with a pH decrease from 6.8 to 7.2.

Dehaji *et al.* (2012) analyzed the symbiotic efficiency of 47 *Rhizobium* strains with 6 common bean varieties under greenhouse condition. In laboratory tests, the ability of fourteen superior strains to solubilize phosphate, zinc and generate auxin, HCN and siderophores was assessed. On solid medium, none of the isolates could solubilize ZnO or ZnCO₃, although in liquid medium, several of them had negligible solubilization. Strains Rb113 and Rb130 had the maximum P and Zn solubility in liquid and solid media, respectively. Rb102 was the strain that produced the most siderophores.

Aarab *et al.* (2015) investigated the phosphate solubilization activity of four *Pseudomonas* (PP7, PP17, PP31and PE15) strains isolated from rice rhizosphere in a variety of media, including NBRIP (National Botanical Research Institute's phosphate growth medium), YED (Yeast Extract + Dextrose), Pikovskaya medium amended with 0.5 per cent TCP and modified PVK medium containing CaHPO₄, FePO₄, AlPO₄ and Ca₅HO₁₃P₃ as when FePO₄ and AlPO₄ were used as P sources, all of the strains were able to dissolve calcium phosphates, but none of them were surrounded by transparent halos. P release in PVK substituting glucose for sucrose and galactose was found to be

highest in the presence of glucose, while it didn't exceed 43.64 µg / ml and 19.19 µg / ml in the presence of galactose and sucrose, respectively.

Panda *et al.* (2016) discovered that bacterial strains isolated from the rhizosphere of maize, rice, ginger and large cardamom growing in different parts of Sikkim have phosphate solubilization activity. P-solubilization by isolates from the maize rhizosphere ranged from 60 to 150 per cent (F6, 14 = 140.75, P0.05), with isolate M71 having the highest solubilization. Similarly, the solubilization efficiency of rice isolates ranged from 75 to 160 per cent (F7, 16 = 129.06, P0.05), with R71 demonstrating the highest efficiency. PSE of ginger and big cardamom isolates ranged from 46 to 140 per cent (F5, 12 = 140.36, P0.05) and 80 to 150 per cent (F4, 10 = 62.68, P0.05), respectively. R71 from the rice rhizosphere had the highest solubilization efficiency of nearly 160 per cent while among the maize isolates, M61 was determined to be the best at solubilizing tri calcium phosphate (203.7 mg L^{-1}) and M28 had the lowest (55.2 mg L^{-1}) solubilization efficiency. The rice isolate R31 had the highest P-solubilization (169.1 mg L^{-1}), while R33 had the lowest (30.2 mg L^{-1}). G49 (193.5 mg L^{-1}) and G15 (63.4 mg L^{-1}) had the highest and lowest P-solubilization, accordingly, for ginger isolates, whereas big cardamom isolates had the highest P-solubilization of 196.2 mg L^{-1} in (C61) and the lowest of 42.1 mg L^{-1} in (C31).

Pande *et al.* (2017) discovered that three isolates, A4, C1 and H6, have phosphate solubilization activity, with C1 being identified as *Burkholderia cepacia* and A4 and H6 being identified as *Alcaligenes aquatilis*. In Pikovskaya agar plates, all three isolates had a phosphate solubilizing index (PSI) of 4.0, with C1 (4.88 ± 1.69) having the highest PSI, followed by H6 (4.64 ± 1.12) and A4 (4.48 ± 0.30). C1 ($305.49 \pm 10.72 \text{ g/ml}$) had the highest P release in pikovskaya's broth containing 5000 mg/ml tricalcium phosphate, followed by H6 ($282.38 \pm 11.81 \text{ g/ml}$) and A4 ($277.72 \pm 1.45 \text{ g/ml}$) in 8 days, with a decline in pH of culture medium (3.08 ± 0.08) from an initial pH of 7.11 and C1 (3.08 ± 0.08) having the lowest pH and further followed by A4 (3.36 ± 0.11) and H6 (3.82 ± 0.12).

Mumtaz *et al.* (2017) reported that, a number of pure rhizobacterial colonies were isolated from maize rhizosphere and screened for their ability to solubilize zinc oxide. These isolates were screened on the basis of zinc and phosphate solubilization, IAA production, protease production, catalase activity and starch hydrolysis. All the selected isolates were also positive for oxidase activity (except ZM22), HCN

production (except ZM27) and utilization of citrate. More than 70% of isolates produces ammonia, hydrogen cyanide, siderophores, exopolysaccharides and cellulase. More than half of isolates also showed potential for urease activity and production of lipase. The ZM31 and S10 were the only isolates which showed the chitinase activity.

Cavite *et al.* (2018) explored the phosphate solubilization activity of 25 rice rhizosphere isolates. Seven of the 25 isolates produced varying lengths of colony diameters as well as halo zone diameters and their phosphorus solubilization index (PSI) ranged from 1.25 to 1.60 mm. Isolate IBBY1(1.60) had the highest efficiency, while isolate IBBY1b had the lowest efficiency (1.25).

Gupta *et al.* (2022) performed screening for phosphate solubilization activity revealed that the halo zone diameter formed by these isolates extended from 2.1 to 3.2 mm. The phosphate solubilizing efficiency (SE) ranged from 35.4 to 50.9, with PS4 recording the highest value of 50.9 and the highest P solubilization of 10.22 g/ml on the 7th day. The phosphorus solubilization potential of these identified PSRB strains can be used and investigated in the phosphorus-deficient rice growing areas of Jammu.

2.4 Screening of isolates for solubilising activity using various P and Zn sources

Desai *et al.* (2012) tested in vitro for the solubilization of 'Zn' and 'Pi' from insoluble zinc (ZnO , $ZnCO_3$) and phosphorus (TCP), respectively through the strains of *Azotobacter* (31), *Azospirillum* (38), *Bacillus* (19) and *Pseudomonas* (82) that were obtained from a variety of crop production systems. 15 strains solubilized zinc and generated $>50\text{ cm}^2$ solubilization zones on solid media after 15 days of incubation. In broth culture, B116 could release the most accessible zinc (13.12 ppm) with ZnO as the zinc source, whereas B118 could release the most available zinc with $ZnCO_3$ (16.3 ppm). Except in the case of B116, *Pseudomonas* strain PIII-105 emitted the greatest accessible 'Pi' (14.8 ppm) and solubilization of Zn and Pi correlated with a decrease in medium pH. Except in the case of B116, *Pseudomonas* strain PIII-105 emitted the greatest accessible 'Pi' (14.8 ppm) and solubilization of Zn and Pi correlated with a decrease in medium pH. With both Zn sources, two (2) strains of *Azospirillum*, six strains each of *Bacillus* and *Pseudomonas* demonstrated a clearance zone area of $>50\text{ cm}^2$. TCP was soluble in two strains of *Azospirillum* and *Bacillus* and three strains of *Pseudomonas*. These Zn and P sources were solubilized by *Azospirillum* strains As-20

and As-22, *Bacillus* strains B113 and B118 and *Pseudomonas* strains P17, P33 and PIII 105, demonstrating their ability to supply both important minerals to plants.

Peter *et al.* (2014) tested indigenous phosphate solubilizing *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* isolated from Bamboo plant rhizosphere for abiotic stress tolerance of temperature (10°C, 50°C), pH (3, 5, 7, 9, 11) and NaCl conc. (2.0, 4.0, 6.0, 8.0, 10 %) and in the NBRIP-BPB (National Botanical Research Institute's Phosphate medium with bromophenol blue) medium Both isolates thrived at 10°C, pH 5, 7 and 8 and tolerated NaCl concentrations of 2.0, 4.0 and 6 per cent, respectively, whereas neither grew at 50°C, pH 3, 9, or 11 and NaCl concentrations of 8 and 10 per cent, respectively. They also demonstrated independent PQQ action, HCN and auxin production as plant growth promoters.

Khanghahi *et al.* (2018) investigated the optimal growth conditions for two zinc solubilizing bacteria (ZnSB) that have been examined for use as bioinoculants to overcome Zn deficiency in soils. A laboratory-scale experiment was conducted to assess the potential of 80 plant growth promoting bacteria (PGPB) strains isolated from the rhizosphere of barley and tomato plants to solubilize zinc. To find the most effective ZSB, isolates were tested on Trismineral medium supplemented with 0.1 per cent zinc oxide, zinc carbonate and zinc phosphate individually. After 10 days of incubation at 29°C, two strains (*Agrobacterium tumefaciens* and *Rhizobium sp.*) were chosen based on the presence of a distinct halo zone around their colonies in the solid medium supplemented with zinc oxide. The results of solubilization at various pH values revealed that these strains have solubilization activity in the pH range of 8–10, but no solubilization at pH 6 and 7. At pH 9, the greatest Zn solubilization values were 51.4 mg L⁻¹ (*Agrobacterium tumefaciens*) and 72.1 mg L⁻¹ (*Agrobacterium tumefaciens*) (*Rhizobium sp.*). Different NaCl concentrations have an effect on bacterial growth in vitro, according to the data. For *Agrobacterium tumefaciens* and *Rhizobium sp.*, the salt concentration necessary for 50% absorbance inhibition was 2.11 and 2.27 per cent NaCl, respectively. The greatest bacterial growth was reported at a NaCl concentration of 0.8 per cent.

Bhatt *et al.* (2020) carried out a study to isolate range of zinc solubilizing bacteria (ZSB) and to analyse them for plant growth promotion (PGP) attributes with accordance to *Capsicum annuum L.* Seventy bacteria were obtained from cattle dung and tested for zinc solubilization purpose (ZnO and ZnCO₃). Whereas, due to its

greatest zinc solubilization (ZnO) capabilities, isolate CDK25 was shown to be the most potent (5.0 cm). Atomic absorption spectroscopy (AAS) was used for the quantitative experiment and CDK25 demonstrated much greater ZnO solubilization (20.33 ppm). CDK25 was found to have numerous PGP properties, including phosphate solubilization, phytase formation, indole acetic acid (IAA) and siderophore synthesis. According to a quantitative investigation, isolate CDK25 can solubilize and produce far more phosphate (281.59 µg/ml) and IAA (13.8 µg/ml), respectively. Under pot culture assay, ZSB was applied to several treatments, with T3 (seeds+CDK25) having the greatest impact on plant development metrics and the highest zinc level in fruit (0.25 mg/100 g). As a zinc solubilizer, PGP strain and *Bacillus megaterium* based on 16S rRNA gene sequencing, isolate CDK25 has the maximum potential throughout the studies. As a result, careful application of this bacteria could help provide enough amounts of soluble zinc, as well as improved plant growth, nutrient uptake and yield in a long-term way.

Jagana *et al.* (2019) isolated ZnSB from brinjal crops rhizospheric soil, the rhizospheric samples were collected from 12 different locations in north Kashmir districts their screening and characterization was carried out. Insoluble zinc was obtained from zinc carbonate. Out of 80 Zn solubilizers, ten of the most promising isolates were kept for additional mineral solubilization testing (Zn and K). Znsb (Kup) M2, which was identified as *Bacillus cereus* using molecular methods, had the highest solubilization index (S.I) and solubilization efficiency (S.E) at 48 and 72 hours, respectively. The isolate *Bacillus cereus* Znsb (Kup) M2 emitted the greatest zinc level of 12 ppm/ml in broth containing zinc carbonate 10 days after incubation. *Bacillus cereus* had the greatest index of 1.28 and 1.66 for IAA activity, potassium solubilization and antagonism against *Colletotrichum capsici*, with *Bacillus cereus* having the best value of 1.28 and 1.66.

2.5 Effect of P-Zn isolates on available nutrient content in soil

Khan *et al.* (2009) studied the recent developments on microbial P solubilization and mentioned that phosphorus solubilizing bacteria play role in phosphorus nutrition by enhancing its availability to plants through release from inorganic and organic soil P pools by solubilization and mineralization. Principal mechanism in soil for mineral phosphate solubilization is lowering of soil pH by microbial production of organic acids and mineralization of organic P by acid phosphatases. Use of phosphorus solubilizing

bacteria as inoculants increases P uptake. These bacteria also increase prospects of using phosphatic rocks in crop production.

Vyas and Gulati (2009) investigated the effects of diverse *Pseudomonas* strains, including *P. trivialis* (BIHB 745), *P. trivialis* (BIHB 747), *Pseudomonas sp.* (BIHB 756) and *P. poae* (BIHB 808), on maize growth and total N, P and K content in single super phosphate treatments. In maize, strains produced significantly higher or statistically equivalent growth and total N, P and K content than a single superphosphate treatment. NP_{TCP} K+ Pt (BIHB745) had the highest accessible P content among PSB treatments and NP_{ssp} K had the highest available K content among uninoculated treatments, while NP_{TCP} K+ Psp had the most available K. (BIHB 756).

Chakraborty *et al.* (2010) reported that isolated PSB strains solubilized P from calcium phosphate to a greater amount than rock phosphate, aluminum phosphate and iron phosphate.

Duarah *et al.* (2011) reported that inoculation with PSB- *Erwinia tasmaniensis* TP08, *Pseudomonas aeruginosa* TP16 and *Pseudomonas aeruginosa* TP373 increased the availability of P in the soil of *Oryza sativa* L. plant by 10%, 1.5% and 10% respectively.

Hridya and Byju (2014) conducted an on-farm experiment to investigate the effect of NPK fertilizers, bio control agents (*Trichoderma* and *Pseudomonas fluorescens*) and biofertilizers (Azospirillum, AM fungi and phosphorus solubilizing bacteria) on soil chemical and biochemical properties in cassava-growing Vertisols during 2008 and 2009. The experiment used a split plot design, with two amounts of NPK fertilizer as main plot treatments and eight microbial inoculation treatments as subplot treatments. By reducing the amount of NPK, *Azospirillum* with *Trichoderma* (170.58 kg/ha), AM fungi with *Trichoderma* (57.85 kg/ha) and *Trichoderma* alone (473.70 kg/ha) increased accessible nitrogen, phosphorus and exchangeable potassium. At a 50 per cent suggested NPK rate, *Pseudomonas fluorescens* plus *Trichoderma* enhances the available iron in soil. In general, microbial inoculations at 50% of the indicated rate produced comparable or better outcomes than uninoculated controls at the prescribed NPK rate.

Hassan and Bano (2015) recovered *Pseudomonas moraviensis* and *Bacillus cereus* from the rhizosphere soil of the Khewra salt range halophytic weed (*Cenchrus*

ciliaris L.) and employed them as bioinoculants. After seed germination, aqueous solution of tryptophan was added to the rhizosphere soil at 1mg/L and plant growth promoting rhizobacteria (PGPR) were treated to wheat (*Triticum aestivum*) via seeds soaking. For two years, the experiment was conducted at Quaid-e-Azam University in Islamabad, in both pots (filled with sterilized soil) under axenic conditions and in the field under natural conditions. *Pseudomonas moraviensis* and *Bacillus cereus* inoculation improved soil organic matter P, K, Ca and availability considerably. Inoculation with these PGPR had a favourable effect on growth. By increasing the number of seeds per spike and spike length with both PGPR, the number of plants at yield and seed establishment increased. In potted plants, the effects of PGPR inoculation alone and with tryptophan were more evident. The findings suggest that tryptophan supplementation is a viable option for enhancing PGPR capacity and thereby improving wheat growth and physiology.

Dinesh *et al.* (2018) devised experiments to assess the Zn solubilization capacity of a variety of bacterium isolates. Because of its improved Zn solubilization in vitro, ZnSB2 (*B. megaterium*, KY687496) was judged to be the most promising of the six promising Zn solubilizing bacteria (ZnSB). At all sampling days, the net Zn solubilized by ZnSB2 was considerably higher than that solubilized by the other ZnSB in the quantitative investigation. In the green house, ZnSB2 was further evaluated with turmeric as the test crop. ZnSB2 was used alone or in conjunction with the chemical Zn (75 per cent and 100 per cent of recommended Zn). At 120 days after planting, the soil available Zn level in the 75 per cent Zn + ZnSB2 treatment ($12.69 \pm 2.96 \text{ mg kg}^{-1}$) was on par with the level in the 100 per cent Zn treatment ($12.74 \pm 2.63 \text{ mg kg}^{-1}$), while at harvest, the 75 per cent Zn + ZnSB2 treatment maintained 65.0 per cent higher available Zn levels than the 100 per cent Zn treatment. ZnSB2 had a beneficial influence on rhizome yield, which was comparable in treatments with ZnSB2 + 75 per cent Zn (154.2 g $\pm 36.0 \text{ pot}^{-1}$) and ZnSB2 + 100 per cent Zn ($177.2 \pm 36.7 \text{ g pot}^{-1}$). The ZnSB2 strain was identified as a possible candidate for improved Zn solubility in soil, allowing for lower inorganic Zn application rates.

Soil Enzymes activity

Hridya and Byju (2014) conducted an on-farm experiment to investigate the effect of NPK fertilizer's, bio control agents (*Trichoderma* and *Pseudomonas fluorescens*) and biofertilizers (Azospirillum, AM fungi and phosphorus solubilizing

bacteria) on soil biochemical and microbial biomass carbon in cassava-growing Vertisols during 2008 and 2009. The experiment used a split plot design, with two amounts of NPK fertilizer as main plot treatments and eight microbial inoculation treatments as subplot treatments. All cultures had enhanced their soil dehydrogenase and glucosidase enzyme activity by applying 50 per cent of the recommended NPK rate. The interaction effect revealed considerably greater microbial biomass carbon in AM fungi when *Trichoderma* was applied at 50% of the prescribed NPK rate (3792.45 g/g soil), which was comparable to soil treatment of all cultures at 100% and 50% of the recommended rate.

Stephen *et al.* (2015) investigated the effect of phosphate solubilizing isolates PSB 12 and PSB73, which were identified as *Gluconacetobacter sp.* (MTCC 8368) and *Burkholderia sp.* (MTCC 8369), respectively, on the growth of rice plants (Jyothi PTB 39) in pot conditions. After 60 days of experiment, the treatment with the mixed inoculation of *Gluconacetobacter sp.*+ *Burkholderia sp.*+ RP60 produced the highest nutrient uptake and yield and the treatment with the *Gluconacetobacter sp.*+ RP60 retained the highest phosphates activity ($30.96 \mu\text{g TPF g}^{-1}$) and the treatment with the *Gluconacetobacter sp.* + *Burkholderia sp.*+ RP₆₀ retained the maximum dehydrogenase activity ($38.88 \mu\text{g TPF g}^{-1}$).

Raghuveer *et al.* (2017) investigated the influence of PSB strains *Arthrobacter sp.*, *Enterobacter sp.*, *Bacillus polymyxa*, *Pseudomonas sp.* and 4 phosphorus levels (0, 20, 40, 60 kg P₂O₅ ha⁻¹) on soil microbial and enzymatic activity (cv. Shahsarang) in a field experiment with a local high yielding rice cultivar. At 60 kg P₂O₅ ha⁻¹ dosage, soil microbial carbon (C) increased by (454.05 g^{-1} soil) and dehydrogenase activity (DHA) was also found to be significantly greater ($5.61 \text{ g TPF g}^{-1} \text{ h}^{-1}$) than at other doses of P. At 60 kg P₂O₅ ha⁻¹ and with *Pseudomonas sp.*, there was a substantial interaction effect on soil microbial C as well as DHA activities (481.19 g g^{-1} and $5.03 \text{ g TPF g}^{-1} \text{ h}^{-1}$). Phosphates activity (PHA) was also higher with P control ($37.15 \text{ g PNP g}^{-1} \text{ h}^{-1}$) and with *Pseudomonas sp.* inoculation ($35.14 \text{ g PNP g}^{-1} \text{ h}^{-1}$) and with a significant interaction of P control and *Pseudomonas sp.* for PHA.

Sreelakshmi *et al.* (2019) investigated the impact of different P doses (100, 75, 50 and 25 per cent) and P solubilizers (AMF, *Pseudomonas* and *Bacillus*) on the activity of different enzymes and microbial parameters in soil, as well as their impact on the growth and yield of the test crop tomato (var. Vellayani Vijai). At over four

monoammonium phosphate, PSB showed a rising tendency in dehydrogenase activity. The highest value of 59.89 µg of p nitro phenol released/g of soil 24/h was found to be in treatment comprising of PSB at 4 MAP. The highest value of MBC ($380 \mu\text{g}^{-1}\text{g}$ soil) was registered in T9 (25% P + AMF) whereas the treatment T5 (50% P + PSB) recorded the maximum MBP content of ($71.83 \mu\text{g}^{-1}\text{g}$ soil).

Nutrient Uptake

Babhulkar *et al.* (2000) revealed that nitrogen uptake was significantly enhanced by application of zinc up to 15 kg Zn ha^{-1} in safflower crop.

Brennan *et al.* (2006) compared the responses of cool-season legumes to zinc application and found that faba beans used the indigenous soil zinc more effectively than chickpea and lentil.

Ayalew (2016) investigated the impact of zinc fertilization on haricot bean yield and zinc, iron, copper and manganese concentrations in tissues. The results showed that haricot bean yield and tissue micronutrient content differed significantly among different sites (soils). Zinc fertilization had no effect on haricot bean output or tissue concentrations of Fe, Cu and Mn but it did significantly increase leaf and seed zinc concentrations.

Sida-Arreola *et al.* (2017) investigated the effect of zinc bio fortification on the zinc content of common bean under greenhouse circumstances, employing two types of zinc (ZnSO_4 and DTPA-Zinc) at four doses (0, 25, 50 and 100 M) added in a hydroponic system. The findings demonstrated that no matter how low-dose zinc was applied, it raised the concentration in the seed.

Malakooti *et al.* (2017) investigated the effects of soil and foliar zinc and iron applications on soybean quantitative and qualitative features, finding that soil zinc application produced the maximum seed production. The highest biological yield was obtained by combining soil zinc application with foliar iron spray. When zinc was supplied to the soil, seed zinc content rose, but the change was small when compared to foliar zinc supplementation.

Kumar *et al.* (2021) conducted research in the greenhouse and field to establish effective indigenous plant growth promoting (PGP) rhizobacteria consortia for improved plant growth attributes and nutrient uptake by wheat crops. Six efficient isolates were selected from 120 rhizosphere soil isolates based on biochemical analysis.

Three separated efficient PGPR strains, as well as a *Pseudomonas aeruginosa* collected strain, were chosen to prepare distinct bio formulations for improving wheat plant growth and yield in greenhouse and field environments. Tetra combination of *Bacillus megaterium*, *Arthrobacter chlorophenolicus*, *Enterobacter sp.* and *Enterobacter spp.*, *P. aeruginosa* showed significant increases in plant height (24.56 per cent and 47.06 per cent), grain yield (75.80 per cent and 40.09 per cent) and straw yield (76.55 per cent and 42.63 per cent) when compared to the control. Similarly, tetra- and tri-inoculations caused wheat to acquire considerably more test weight and nutrients than the control. Tetra-inoculation of *B. megaterium*, *A. chlorophenolicus*, *enterobacter spp.* and *P. aeruginosa* strains can thus be used as an effective PGPR consortium for increased wheat yield.

Yield attributes

Kumar *et al.* (2011) screened the *Staphylococcus* isolates for antagonistic activity against *Sclerotium rolfsii* and *Colletotrichum capsici* and plant growth promoting traits. Out of all isolates 57.5% isolates solubilized phosphate.

Jha *et al.* (2012) analyzed the consequences of single and dual inoculations of four phosphate solubilizing strains *Pseudomonas fluorescens* (BAM-4), *Burkholderia cepacia* (BAM-6), *B. cepacia* (BAM-12) and *Aeromonas vaga* (BAM-77) along with TCP on growth of mungbean plants in pot experiment. Overall, the best single inoculants (with and without TCP) to be observed as *Aeromonas vaga* (AV) and *Pseudomonas fluorescens* (PF). Among combinations with *P. fluorescens*, PF + AV (with TCP) and PF + BC1, PF +BC2 (without TCP) were observed to be the best combinations for enhancement of growth parameters in maize.

Deshwal and Kumar (2013) studied the impact of plant growth promoting (PGP) activity of *P. aeruginosa* (PW-99), *P. aeruginosa* (PW-136), *P. putida* (PW-2), *P. putida* (PW-56), *P. cepacia* (PW-18), *P. cepacia* (PW-43), *P. fluorescens* (PW-5), *P. fluorescens* (PW-104) strains on rice crop grown in pot experiment. When compared to control, *P. fluorescens* (PW-5) produced the most shoot, root and dry weight of mung bean plants (157.72 per cent, 408.06 per cent and 233.84 per cent, respectively).

Erdal and Kucukyumuk (2013) investigated the effects of phosphorus and zinc on lentil grain production and several characteristics affecting zinc bioavailability, concluding that zinc fertilization resulted in lower grain P concentrations and related

metrics. Increases in both Zinc and protein concentrations in grains decreased phytic acid/Zinc molar ratios; however, phytase activity was negatively affected by increases in both Zinc concentrations in grains. Zinc fertilisation enhanced grain yield.

Joshi *et al.* (2013) reported that enhancement of plant growth parameters and 31% increase in grain Zn over control was observed during application of bacterial isolates and their consortium with $ZnSO_4 \cdot 7H_2O$ @ 5mM to wheat, in a pot experiment.

Reetha *et al.* (2014) used a pot culture experiment using sterilized air dried soil and viable onion seeds to investigate the effects of *Pseudomonas fluorescens* and *Bacillus subtilis* on onion plant growth. On onion plants, both bacteria increased root length, shoot length, root and shoot fresh and dry weights over control.

Viruel *et al.* (2014) used a pot experiment to investigate the effects of *Pseudomonas tolaasi* IEXb and *Pseudomonas koreensis* on maize plant growth metrics in both controlled and field environments. Plants treated with *Pseudomonas tolaasii* (IEXb) had a substantial increase in plant height (45%) and shoot dry weight (40%) compared to the uninoculated control under controlled conditions and *Pseudomonas koreensis* (SP28) similarly increased P content. Under field conditions, the IEXb strain was evaluated in combination with triple superphosphate (TSP) as a P fertilizer and the IEXb strain increased seedling emergence by 8%, shoot length by 19%, grain yield by 44%, 1000-grain weight by 18%, total dry biomass by 32%and P content by 56% in maize plants, revealing *P. tolaasii* (IEXb) as an effective bio inoculant with TSP.

Linxi (2015) carried out two year field trial to analyze the influence of increasing zinc (Zinc) fertilisation rates on zinc accumulation content in winter wheat grown in soils without zinc deficiency and discovered that zinc concentrations in various wheat were significantly improved and had positive correlations with zinc fertilizer rates.

Alam *et al.* (2021) conducted an experiment to assess the potential of phosphate solubilizing bacteria (with and without PSB) in enhancing wheat growth and yield at various P levels (0, 25, 50and 100 per cent of recommended P). When compared to the remainder of the treatment combinations, the PSB with 100 per cent prescribed P considerably improved wheat tillers m^{-2} , grains spike $^{-1}$, grains and biological yield. The efficacy of PSB and full prescribed P was further proven by a considerable increase in 100 grain weight and photosynthetic rate furthermore, compared to wheat yield

qualities without PSB. PSB were beneficial in optimizing wheat yield attributes at respective P levels. Data suggest that using PSB in combination with the 100 per cent prescribed P as inorganic phosphorus can improve wheat growth and yield over sole application of P fertilizers or PSB.

Yadav *et al.* (2021) revealed that the different groups of microbes like *Methylobacterium*, *Paenibacillus*, *Pseudomonas*, *Rhizobium*, *Serratia* and *Staphylococcus* present in the soil naturally plays a several significant roles like nutrient cycling, maintenance of soil fertility and decomposition of organic matter. They reported that these microbes can be used as in agriculture as plant protector, their biotechnological contribution potential for nutrient cycling, plant growth improvement, nutrient uptake and plant growth enhancer.

Materials and Methods

Chapter 3

Materials and Methods

The present investigation entitled “**Isolation, Identification and Evaluation of phosphate and zinc Solubilizers in wheat rhizosphere**” was conducted during 2021-2022 in the Division of Soil Science and Agricultural Chemistry of Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu (J&K). Materials and methods adopted during the investigation have been presented in this chapter under the following heads:

3.1 Soil sampling

A total of thirty two soil samples were collected from the wheat rhizosphere from two districts viz., Udhampur and Jammu of Jammu divisions of J&K UT based on GPS location for assessing variability in soil properties. For this purpose, the shoot portion was cut off after the plants were uprooted carefully and the rhizospheric soil (0-15cm) deep along with roots of uprooted plants was aseptically collected in small polythene bags and brought to the laboratory and stored at 5° C before processing.

3.2 Isolation of phosphate and zinc solubilizers

PZn and ZnP isolates were isolated using nutrient agar medium and further culturing on selective medium from rhizosphere soil of wheat crop at tillering and earhead initiation stage by using serial dilution technique.

3.3 Biochemical identification of isolates

Biochemical characteristics were used to distinguish pure cultures (Sneath-Peter *et al.* 1994). HiMedia biochemical identification kit uses a single-step inoculation technique that leads to the final identification of the test organism under investigation. The tests are based on principles of the pH change and substrate utilization. Organisms undergo metabolic changes during incubation, which are observed by a colour change in the media that can be seen by the expansion of a reagent. By using KB002 HiAssorted™ Biochemical test, the likely positive phosphate solubilizing bacteria and zinc solubilizing isolates were characterized using biochemical tests.

3.3.1 Biochemical characterization

For biochemical characterization, bacterial inoculums were prepared by inoculating individual colonies in sterilized test tubes containing 15 ml of PVK and ZnS broth and incubated at 37°C for 6 hrs.

The kits were opened aseptically and each well was injected with 50 µl of inoculum by surface inoculation and incubated for 24 hours at 37±1°C. The biochemical characterization, was performed for various tests viz., Citrate utilization, Urease, Nitrate reduction and H₂S production and subjected to identification based on classification as outlined in Bergey's Manual of Systematic Bacteriology.

3.4 Mechanisms of phosphate and zinc solubilization

3.4.1 Qualitative Method

For qualitative estimation of phosphate solubilization, petri plates containing PVK agar media were subjected to treatment with 1% TCP and a loop of each bacterial culture was added to each hole. Plates were incubated at 28±2° C for 72 hrs. and observed for clear zone around the hole. After inoculation halo zones were observed around colonies which were measured at 7th and 9th day of incubation and solubilization efficiency and solubilization index were calculated by using the following formula (Sunithakumari *et al.* 2016).

For qualitative estimation of zinc solubilization, Petri plates containing ZnS agar media were prepared and a loop of each bacterial culture was added to each hole. Plates were incubated at 28±2° C for 48 h and observed for clear zone produced around the well. After inoculation halo zones were observed around colonies which were measured at 7th and 9th day of incubation. SI and SE were calculated by using the formula given by Sunithakumari *et al.* 2016.

3.4.2 Quantitative Method

For quantitative estimation of phosphate solubilization PVK broth and ZnS broth were prepared and absorbance was measured at different days of incubation against KH₂PO₄ with 25 ml Barton's reagent to make volume up to 50ml. After 10 minutes absorbance of the resultant colour of the filtrate was measured in spectrophotometer at 420 nm and 660 nm wavelength.

For quantitative estimation of zinc solubilization, Zinc solubilizing broth and PVK broth was prepared and 10 µl of bacterial culture was inoculated into each flask containing ZnS broth and PVK broth. The flasks were incubated at 28°C for 13 days on incubator shaker at 150 rpm. At 13th days of incubation, culture broth was centrifuged at 10,000 rpm for 10 min. Concentration of Zn in supernatant was measured with atomic absorption spectrophotometer.

3.4.3 Change in pH value of culture medium

Under aseptic conditions, isolates were inoculated using sterilized inoculation loop and incubated at 37° C and pH was measured in successive intervals.

3.5 General Description of the Study Area

3.5.1 Experimental Location

Pot experiment was conducted at Division of Soil Science and Agricultural Chemistry of Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu (J&K). The experiment was laid out on Rabi season wheat (*Triticum aestivum* L.) crop in the sub-tropical zone at latitude of 32.65° North and longitude of 74.80° East and an altitude of 293 m above sea level.

3.5.2 Experimental Details

The experiment was laid out on Rabi season wheat (*Triticum aestivum* L.) crop (variety HD-2967) planted in pots. The number of treatment combinations were 15, which were replicated four times with recommended dose of NPK, TCP and ZnCO₃ along with broth culture of PSB (Phosphate solubilizing bacteria) and ZnSB (Zinc solubilizing bacteria) strains were tested in the experiment. All other cultural operations were uniformly practiced in the experiment.

3.5.3 Climate

The experimental area Chatha (Jammu) falls in sub-tropical zone of Jammu and Kashmir (UT). The area receives an annual precipitation of about 1200mm, most of which is received as monsoon rains during July to September (about 70%). The mean maximum temperature, minimum temperature, Rh (M) and Rh (E) remained during the investigation were 24°C, 8.9°C, 87% and 50% respectively. The highest temperature during the year does not ordinarily exceed 48°C in summers and the minimum does not

go below 4°C in winter months. Detailed information about the weather during experiment in given below in fig. 3.1.

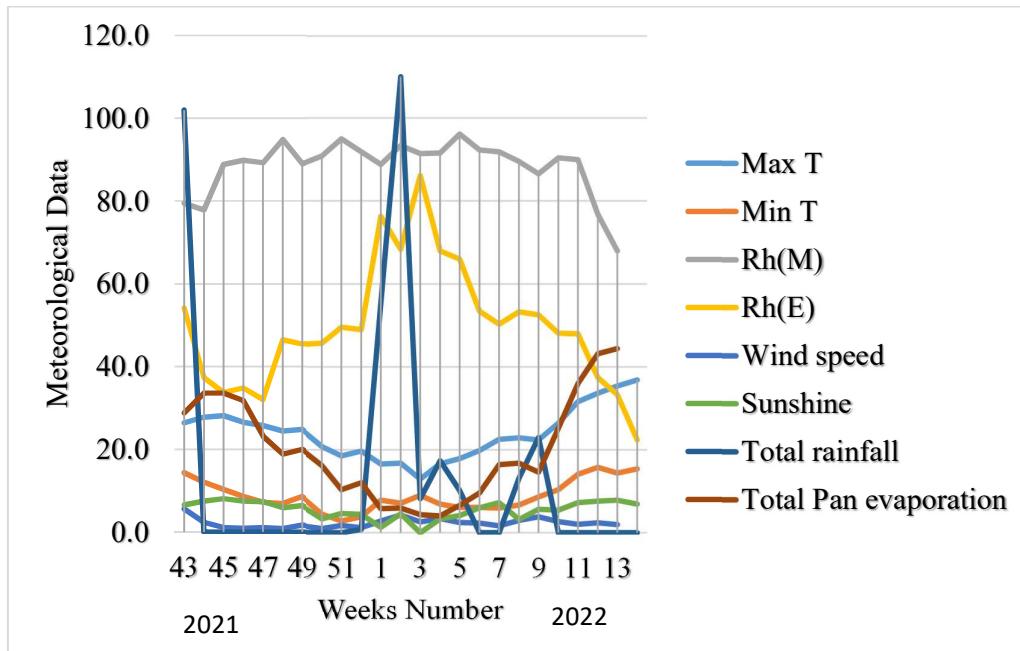


Fig 3.1: Weekly weather data during investigation period.

3.6 Details of Pot Experiment

3.6.1 Collection site of soil samples

Bulk surface soil was collected from the field research station of Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha Jammu located in the sub-tropical zone at latitude of 32.65° North and longitude of 74.80° East and altitude of 293 m above sea level.

3.6.2 Design and Layout of Experiment

The present experiment was laid out in factorial CRD consisting of fifteen treatment combinations and the details of treatment combinations are given below, which were replicated four times. Eight kg each of bulk surface soil was placed in pots of dimensions $30*26*17\text{ cm}^3$.

3.6.3 Treatment Details

Factor 1 (Zinc and Phosphorus)

P₀: Control

P₁: With TCP

P₂: With ZnCO₃

Factor II (Isolates)

B₀: Without isolates

B₁: Isolate 1

B₂: Isolate 2

B₃: Isolate 3

B₄: Consortium (Isolate 2+ Isolate 3)

3.7 Experimental Materials

3.7.1 Plant Material

Vigour and healthy seeds of wheat (HD-2967) were collected from seed processing center SKUAST main campus Chatha, Jammu and surface sterilized with acidic alcohol (H₂SO₄: ethanol, 7:3, v/v) for three minutes further 2-3 repeated washing with distilled water was carried out thoroughly (Sindhu *et al.* 1999). Then inoculation of surface sterilized seeds (six each) was done with broth culture of different isolates and left it to be adsorbed for forty five minutes (Plate 3.1). Six seeds per pot was sown and kept for germination in experimental pots.



Plate 3.1 Seeds soaking in broth culture prior to sowing.

3.7.2 Addition of TCP, ZnCO₃ and Broth Culture

During the time of sowing broth culture of identified isolates was mixed with 8 kg of soil as per treatment combination. TCP (1%) and ZnCO₃ (1%) was applied in 3 split doses at 15 DAS, 45 DAS and 65 DAS respectively as per treatment combinations.

3.7.3 Fertilizer application as per recommended dose for wheat

The recommended dose of urea, DAP and MOP was applied (in grams) in 8 kg of soil per pot (given in table below). The entire dose of DAP+MOP and 50% of Urea was applied as basal dose at sowing time and remaining 50% of Urea was applied in the standing crop plants in two equal doses, first at CRI stage and second at ear head initiation stage.

Fertilizer source	Recommended Dose (Kg/ha)	Dose applied in 8 kg of soil pot⁻¹ (in grams)
Urea (N)	120	0.930
DAP(P ₂ O ₅)	60	0.464
MOP(K ₂ O)	40	0.238

3.8 Laboratory analysis of experimental soil and plant samples

3.8.1 Experimental Material

a) Glasswares

Standard borosil brand glasswares *viz.*, petriplates, conical flasks, measuring cylinder, spirit jar, volumetric flask, beaker, glass rod, test tubes, pipettes, coverslips etc.

b) Equipments

The common laboratory equipments such as autoclave, oven, BOD incubator, refrigerator, deep freezer, weighing balance, laminar air flow cabinet, spectrophotometer, Atomic absorption spectrophotometer(AAS), Flame photometer, pH meter, electrical conductivity meter, rotary shaker, etc.

c) Culture media

A. Pikovskaya's agar medium (PVK).

- B. Pikovskaya's Broth
- C. Zinc solubilising Agar medium
- D. Zinc solubilising Broth

3.8.2 Experimental observations

3.8.2.1 Soil analysis

1) Initial status of experimental soil

Before execution of the experiment soil samples collected randomly from bulk surface soil (0-25 cm) were tested for nutrient status and biological properties of soil.

Table 3.1: Initial status of soil of experiment.

Parameter	Values in soil
pH	7.37
Soil texture	Clay loam
OC(g kg^{-1})	4.08
Available N(mg kg^{-1})	83.0
Available P(mg kg^{-1})	7.02
Available K(mg kg^{-1})	75.3
Exchangeable Na (mg kg^{-1})	98.2
Exchangeable Ca (me/100g)	1.06
Exchangeable Mg (me/100g)	0.97
SMBC(μg^{-1} soil)	81.45
Dehydrogenase activity ($\mu\text{g-TPFg}^{-1}\text{soil hr}^{-1}$)	39.7
Phosphatase activity ($\mu\text{g- PNP g}^{-1}\text{soil h}^{-1}$)	30.8
Available Zn (mg/kg)	0.56

2) Collection and preparation of soil samples at crop growth stages for analysis.

Soil samples from each pot out of four replica were collected from 0-15 cm depth from respective treatment of each seedling by uprooting two plants and collecting soil from the rhizosphere at tillering, ear head initiation and harvest stage to study the rhizospheric properties. The soil samples, thus collected, were dried in shade, grounded, sieved through 2 mm plastic sieve and stored in polythene bags. The data on the soil nutrient status and microbiological properties were recorded and the procedures followed are described below table 3.2.

Table 3.2: Analysis method employed for nutrient status and biological properties of soil

Basic properties	Methods Employed	Reference
pH	Blackman's glass electrode pH meter	(Jackson, 1973)
Organic Carbon (g kg^{-1})	Wet digestion method	(Walkley and Black, 1934)
Available Nitrogen (mg kg^{-1})	Alkaline permanganate method	(Subbiah and Asija, 1956)
Available Phosphorus (mg kg^{-1})	0.5 N Sodium bicarbonate (pH 8.5)	(Olsen <i>et al.</i> , 1954)
Available potassium (mg kg^{-1})	Ammonium acetate extraction method (pH 7.0) using flame photometer	(Jackson, 1973)
Exchangeable Sodium (mg kg^{-1})	Ammonium acetate extraction method (pH 7.0) using flame photometer	(Jackson, 1973)
Exchangeable Calcium (me/100g)	EDTA Versenate titration method	(Gupta, 1999)
Exchangeable Magnesium (me/100g)	EDTA Versenate titration method	(Gupta, 1999)
Soil Microbial Biomass Carbon (μg^{-1} soil)	Chloroform fumigation method	Vance <i>et al.</i> , 1987
Dehydrogenase activity ($\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$)	Triphenyl formazon estimation	Casida <i>et al.</i> , 1964
Phosphatase activity ($\mu\text{g- PNP g}^{-1}\text{soil hr}^{-1}$)	p- nitro phenol estimation	(Tabatabai and Bremner, 1969)
Available Zn(mg kg^{-1})	DTPA extraction method	(Lindsay and Norvell, 1978)



Plate 3.2: Pots during sowing of seeds



A) Tilling stage



B) Ear head initiation stage



C) Maturity stage

Plate 3.3: Growth stages of wheat crop in pot experiment

A) Effect of PZn and ZnP isolates on soil physico-chemical properties.

1) pH.

The pH of the soil was measured in 1:2.5 soil water suspension with the help of glass electrode pH meter (Jackson, 1973).

2) Organic carbon (g kg^{-1}).

The soil organic carbon was estimated by Walkley and Black titration method (Wet digestion method, 1934).

B) Effect of PZn and ZnP isolates on soil available nutrients.

3) Available Nitrogen (mg kg^{-1}).

The available nitrogen in soil was estimated by alkaline potassium permanganate method as given by (Subbiah & Asija, 1956).

4) Available Phosphorus (mg kg^{-1}).

The available phosphorus in soil was estimated by Stannous Chloride reduced ammonium molybdate method using Olsen's extractant (Olsen *et al.* 1954) and determined on spectrophotometer at 660 nm wave length.

5) Available potassium (mg kg^{-1}).

1N ammonium acetate was used as extractant and the available potassium was determined by feeding the extract to flame photometer (Jackson, 1973).

6) Available Zinc (mg kg^{-1}).

The available Zn in the soil were estimated by shaking 10 gm. of air dried soil with DTPA extracting solution for 2 hrs. @120 cycles per minute. The suspension was then filtered via whatman no. 42 filter paper and analyzed for zinc with an atomic absorption spectrophotometer (Lindsay and Norvell, 1978).

C) Effect of PZn and ZnP isolates on Exchangeable nutrients.

1) Exchangeable Na (mg kg^{-1}).

1N ammonium acetate was used as extractant and the exchangeable sodium was determined by feeding the extract to flame photometer (Jackson, 1973).

2) Exchangeable Calcium (me/100g).

Ammonium acetate soil extract is titrated with standard EDTA solution using ammonium purpurate as an indicator in the presence of sodium hydroxide solution to note the volume of EDTA used till the colour change is observed from orange red to purple and the amount of calcium is expressed as me/100g.

3) Exchangeable Magnesium (me/100g).

Ammonium acetate soil extract is titrated with standard EDTA solution using Erichrome Black T as an indicator in the presence of NH₄ Cl–NH₄OH buffer to note the volume of EDTA used till the colour change is observed from red to blue and the amount of magnesium is expressed as me/100g.

D) Effect of PZn and ZnP isolates on soil biological properties.

1) Soil Microbial biomass carbon ($\mu\text{g}^{-1}\text{soil}$).

Soil Microbial biomass carbon (SMBC) was determined using the chloroform fumigation extraction methods of Vance *et al.* (1987). A paired set of 20 g fresh soil samples maintained at 4°C were taken, one part was fumigated with ethanol-free chloroform in a desiccator and another was kept under identical conditions without fumigation. Fumigated and non-fumigated samples were then treated with 0.5 M K₂SO₄. The C in the fumigated and non-fumigated extracts was measured and the difference obtained was used to calculate MBC.

2) Dehydrogenase activity ($\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$).

Dehydrogenase activity was determined by monitoring the rate of production of triphenyl formazan (TPF) from tri-phenyl tetrazolium chloride (TTC), using the method of Casida *et al.* (1964).

3) Phosphatase activity ($\mu\text{g- PNP g}^{-1}\text{soil hr}^{-1}$).

The method of determination of phosphatase activity involves colorimetric estimation of the *p*-nitrophenol released by phosphomonoesterases activity using the method of Tabatabai and Bremner (1969).

3.8.2.2 Evaluation of PZn and ZnP isolates on plant properties.

Plant samples from each pot were collected at harvest stage during the first week of April.

E) Effect of PZn and ZnP isolates on nutrient uptake (g pot⁻¹).

1) Nitrogen Uptake

Total nitrogen was estimated by micro-Kjeldhals method as suggested by Jackson (1973).

2) Phosphorus Uptake

Total phosphorus from plant sample was estimated by Vandomolybdate yellow colour method from diacid extract using spectrophotometer by Piper (1966).

3) Potassium Uptake

Total potassium from plant sample was estimated from diacid extract using Flame photometer by Piper (1966).

4) Zinc Uptake

The Zn content in dry matter of wheat grain and straw was determined as per the procedure described by Prasad *et al.* (2006) on Atomic Absorption Spectrophotometer. The Zn content was expressed as mg kg⁻¹. The Zn uptake was calculated by multiplying the grain and straw yields with their respective Zn concentration.

F) Effect of PZn and ZnP isolates on yield attributes.

1) Plant height (cm).

The plant height was measured with the help of metric scale.

2) Number of grains per earhead.

Grains of each earhead from each plant in the pot were counted and averaged.

3) Average spike length (cm).

Spike length was measured from the neck node to the tip of the uppermost spikelet and average length was recorded in cm.

4) Yield (g/pot).

Yield was recorded following threshing and drying. Calculation was done using formula as below:

$$\text{Yield (g/pot)} = \text{Yield obtained from pot} / \text{area of pot}$$

G) Effect of PZn and ZnP isolates on Relative Efficiency

1) Relative Efficiency of Phosphorus Use (REP %)

The relative efficiency of phosphorus use (REP%) was calculated as the ratio between the plant DM (dry mass) under low Pi (Phosphate) and DM (dry mass) under high Pi, as described by (Ozturk *et al.* 2005).

2) Relative Efficiency of Zinc Use (REZn %)

The relative efficiency of zinc use (REZn %) was calculated as the ratio between the plant DM (dry mass) under low Zn input (Zinc) and DM (dry mass) under high Zn input (Ozturk *et al.* 2005).

3.9 Statistical Analysis

The data recorded from laboratory analysis was subjected to statistical analysis using the technique of analysis of variance for factorial completely randomized design for the interpretation of results as described by Gomez and Gomez (1984). The treatment differences were compared at 5 per cent level of significance ($P= 0.05$) using OPSTAT program (Sheoran *et al.* 1998).

Results

Chapter 4

Results

The results observed with respect to different parameters of the study entitled “Isolation, Identification and Evaluation of phosphate and zinc Solubilizers in wheat rhizosphere” have been presented in this chapter under following heads

4.1 Isolation and Biochemical identification of isolates (P&Zn isolates)

- 1) Isolation of P & Zn Solubilizing rhizobacteria.
- 2) Biochemical identification of isolates.

4.2 Mechanisms of Phosphate and Zinc Solubilization by PZn and ZnP isolates in culture medium

- 1) Qualitative estimation of phosphate solubilization.
- 2) Qualitative estimation of zinc solubilization.
- 3) Quantitative estimation of phosphate solubilization.
- 4) Quantitative estimation of zinc solubilization.

4.3 Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ on rhizospheric soil properties

4.3.1 Soil physico-chemical properties

- 1) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil pH at growth stage of wheat.
- 2) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on organic carbon (mg kg⁻¹) at growth stage of wheat.

4.3.2 Soil available nutrients.

- 3) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available N (mg kg⁻¹) status at growth stage of wheat.
- 4) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available P (mg kg⁻¹) status at growth stage of wheat.
- 5) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available K (mg kg⁻¹) status at growth stage of wheat.
- 6) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available Zn (mg kg⁻¹) status at growth stage of wheat.

4.3.3 Exchangeable nutrients.

- 7) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Na (mg kg⁻¹) status at growth stage of wheat.
- 8) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Ca (me/100 g) status at growth stage of wheat.
- 9) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Mg (me/100 g) status at growth stage of wheat.

4.3.4 Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ on biological properties

- 1) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil microbial biomass carbon (μg^{-1} soil)
- 2) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil dehydrogenase activity ($\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$)
- 3) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil phosphatase activity ($\mu\text{g-PNP g}^{-1}\text{soil hr}^{-1}$)

4.4 Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ isolates on plant properties of wheat crop

1) Nutrient uptake (N, P, K & Zn) (g/pot)

2) Yield attributes

- 2.1 Plant height (cm)
- 2.2 Average ear length (cm)
- 2.3 No. of grains earhead⁻¹
- 2.4 Grain and straw Yield (g/pot)

4.5 Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ on Relative Efficiency of Phosphorus Use (REP %) and Relative Efficiency of Zinc Use (REZn %)

4.1 Isolation and Identification of isolates (P&Zn isolates)

1) Isolation of P & Zn Solubilizing rhizobacteria

30 samples were collected from wheat rhizosphere using GPS location, out of 30 samples, 40 isolates were recovered on the basis of halozone formation and three were further screened for biochemical identification based on solubilization potential and coded as (1) PZn-1 isolate (2) PZn-2 isolate and (3) ZnP-1 isolate. The nomenclature was assigned to the isolates on the basis of P and Zn solubilization, first two isolates were P- solubilizers and also exhibited Zn- solubilization ability, whereas third isolate was Zn- solubilizer and exhibited P- solubilization ability.

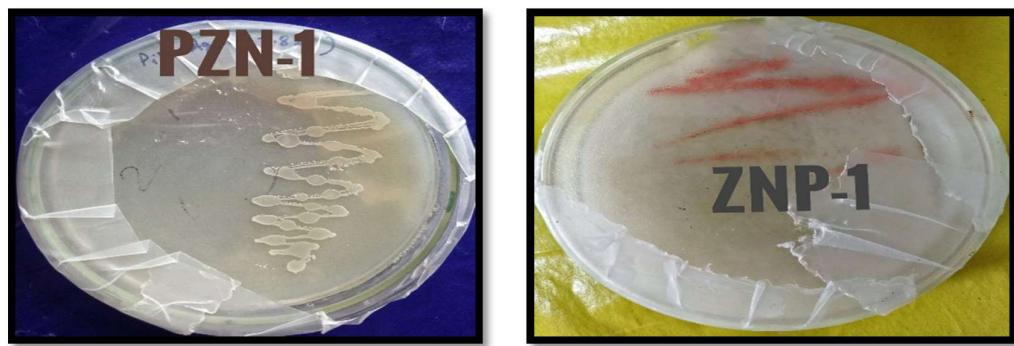


Plate (A)

Plate (B)

Plate 4.1:- Plate A showing PZn -1 isolate on PVK agar and Plate B showing ZnP-1 isolate on ZnS agar.

2) Biochemical identification of isolates.

The PZn and ZnP from wheat rhizosphere soil were characterized for biochemical potential using biochemical kit viz, Nitrate reduction, Urease, Citrate utilization and H₂S production. The observations were recorded at 24 hours, 72 hours and 96 hours and the results are shown in the table 4.1. Based on these biochemical tests performed on isolates, these were subjected to further identification. The tests results were interpreted and positioned in group VI and VIII as outlined in Bergey's Manual of Systematic Bacteriology and confirms the dominance of genera *Staphylococcus*, *Pseudomonas* and *Methylobacterium*.

Table 4.1: Biochemical identification of isolates

Sr. No.	Test	PZn -1	PZn -2	ZnP -1	PZn -1	PZn -2	ZnP -1	PZn -1	PZn -2	ZnP -1
		At 24 hours			At 72 hours			At 96 hours		
1	Citrate Utilization	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Urease	+ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve
3	Nitrate reduction	-ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve
4	H ₂ S production	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

4.2 Mechanisms of Phosphate and Zinc Solubilization by PZn and ZnP isolates in culture medium

1) Qualitative estimation of phosphate solubilization

Data presented in table 4.2 and plate 4.2 reported the phosphate solubilization ability which was determined as clear zone formed around the well at 28°C after 7th and 9th day of incubation on PVK agar. The recovered isolates showed clear zone on culture medium and isolates screened exhibited maximum zone of clearance of varying diameter. The halo zone diameter formed by the isolates ranged from 0.50 cm to 0.65 cm. SI of PZn-2 was found to be highest 0.50 and 0.66 at 7th and 9th day respectively among screened isolates. The phosphate solubilizing efficiency (SE %) ranged from 137% to 166% exhibiting maximum value of 166% by PZn-2 at 9th day. The solubilization efficiency enhanced with incubation period. The diameter of clear halo zone formed by the bacterial isolates increased with the phosphate solubilization efficiency.

**Plate 4.2: Plate showing halozone formation in PVK agar**

2) Qualitative estimation of zinc solubilization

Data pertaining to table 4.2 revealed the zinc solubilization ability which was determined as clear zone formed around the well at 28°C after 7th and 9th day of incubation on ZnS agar. The recovered isolates showed clear zone on culture medium and isolates screened exhibited maximum zone of clearance of varying diameter. The halo zone diameter formed by the isolates ranges from 0.45 to 0.60 cm. SI of ZnP-1 was found to be highest viz. 0.35 and 0.55 at 7th and 9th day respectively among all the isolates. The solubilizing efficiency (SE %) was calculated for the selected isolates which ranged from 112.5 to 155 with highest value of 155 by ZnP-1 isolate. Maximum Zn solubilization was observed at 9th day. The solubilization efficiency enhanced with incubation period. The diameter of clear halo zone formed by the bacterial isolates increased with the zinc solubilization efficiency.

Table 4.2: Qualitative estimation of phosphate and zinc solubilization in culture medium

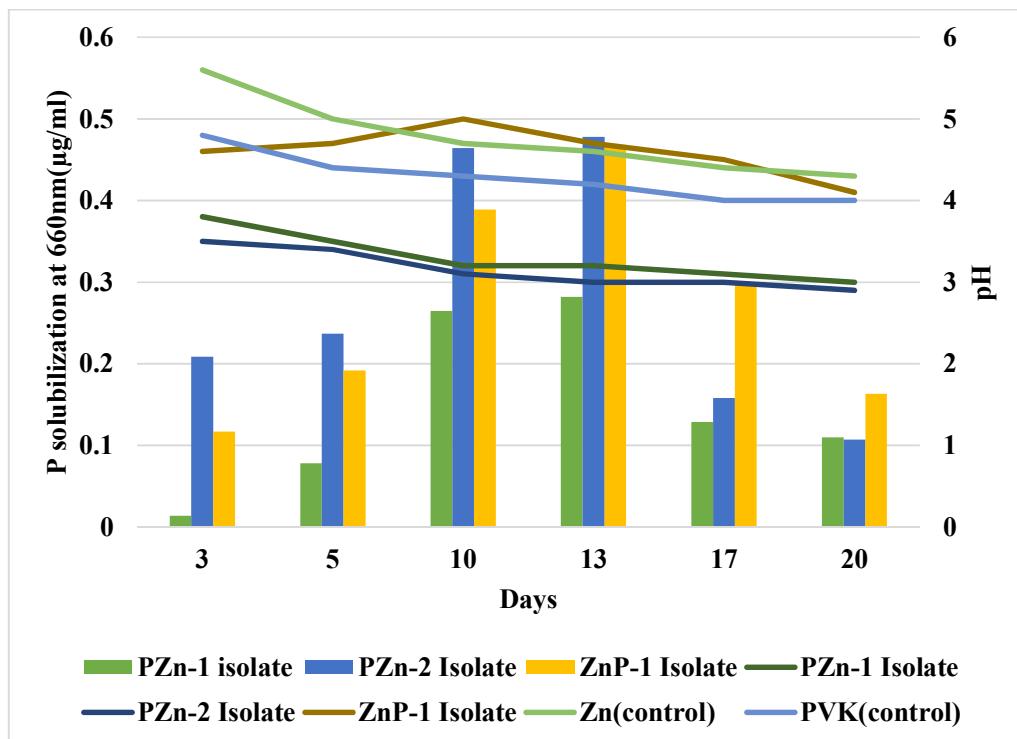
Isolate	PVK Agar		ZnS Agar		PVK Agar		ZnS Agar	
	At 7 th Day				At 9 th Day			
	SI	SE (%)	SI	SE (%)	SI	SE (%)	SI	SE (%)
PZn-1	0.37	137	0.12	112.5	0.50	150	0.25	125
PZn-2	0.50	150	0.20	120	0.66	166	0.30	130
ZnP-1	0.25	125	0.35	135	0.37	137	0.55	155

3) Quantitative estimation of phosphate solubilization in culture broth.

Data presented in table 4.3 and fig 4.1 & 4.2 reported the quantitative estimation of phosphate solubilization in culture medium (PVK broth and ZnS broth) unveiled phosphate solubilization activity in the form of maximum P release in culture supernatant at different days interval. P solubilization by three distinct isolates in the PVK and ZnS broth was analyzed against KH₂PO₄ by using spectrophotometer. Absorbance was measured at 660nm and 420nm wavelengths respectively. The range was recorded in between 0.014µg/ml to 0.651µg/ml. Highest P solubilization i.e., 0.651µg/ml was observed in broth containing PZn-1 isolate at day 13th, after which solubilization showed decreasing trend.

Table 4.3: Quantitative estimation of P solubilization in culture media.

Days	PZn-1 (PVK media)		PZn-2 (PVK media)		ZnP-1(ZnS media)	
	660nm ($\mu\text{g}/\text{ml}$)	420nm ($\mu\text{g}/\text{ml}$)	660nm ($\mu\text{g}/\text{ml}$)	420nm ($\mu\text{g}/\text{ml}$)	660nm ($\mu\text{g}/\text{ml}$)	420nm ($\mu\text{g}/\text{ml}$)
3	0.014	0.070	0.209	0.298	0.117	0.190
5	0.078	0.132	0.237	0.341	0.192	0.234
10	0.265	0.302	0.464	0.528	0.389	0.527
13	0.282	0.346	0.478	0.651	0.468	0.582
17	0.129	0.264	0.158	0.351	0.297	0.300
20	0.110	0.157	0.107	0.247	0.163	0.221
SEM(\pm)	0.02	0.035	0.025	0.03	0.04	0.05
CD at 5%	0.07	0.11	0.08	0.10	0.14	0.17

**Fig 4.1: pH and P solubilization of culture medium at 660nm with successive days of incubation**

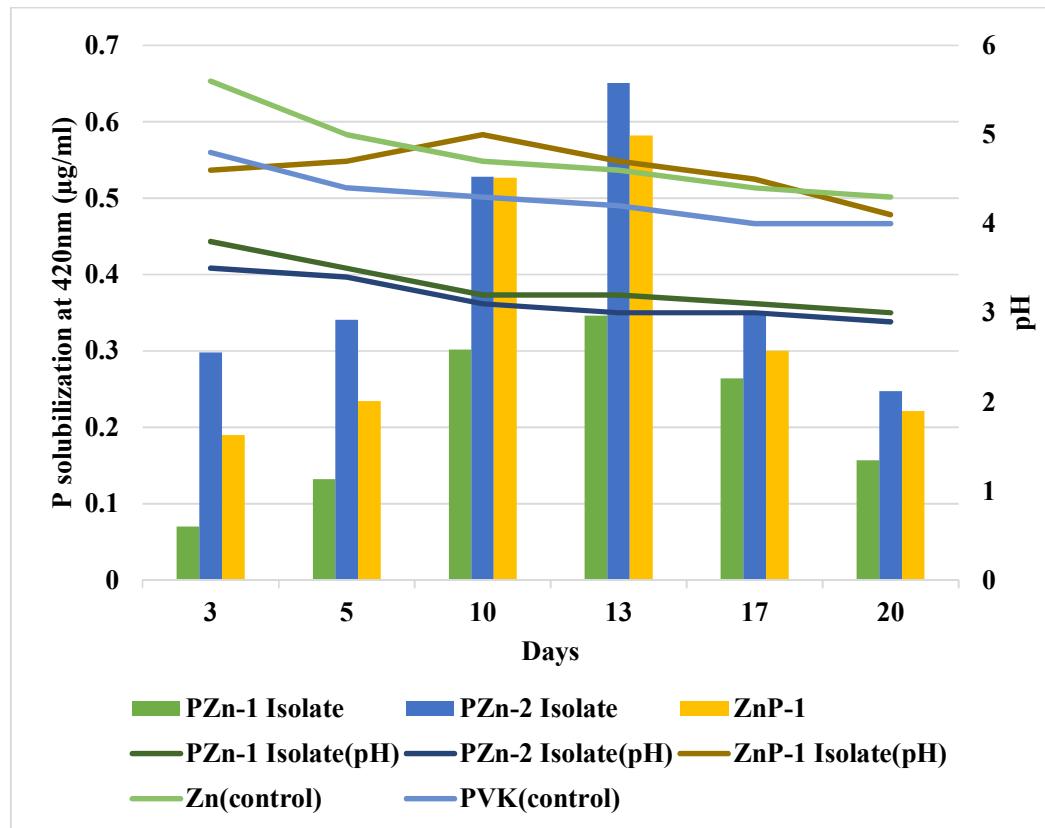


Fig 4.2: pH and P solubilization of culture medium at 420nm with successive days of incubation

4) Quantitative estimation of zinc solubilization in culture medium

The quantitative estimation of zinc content in pikovskaya broth and zinc solubilizing broth was estimated using AAS and a significant difference was observed as compared to control at 7th & 9th day in PVK broth and at 13th day in ZnS broth respectively. The range was recorded viz. 2.50 to 8.25 µg/ml and 3.90 to 9.50 µg/ml at 7th and 9th day respectively and in ZnS broth the range was recorded viz. 37.75 to 41.00 µg/ml and 39.87 to 42.25 µg/ml at 10th and 13th day respectively. The highest Zinc solubilization was found to be in ZnP-1 isolate and lowest was observed in PZn-1 isolate. Growth response was monitored to understand the behavior of bacterial growth phase for a period of one month and data for successive days is presented in table 4.4. The maximum growth was observed at 7th and 9th day (Table 4.4) respectively in PVK broth and at 10th & 13th day in ZnS broth respectively.

Table 4.4: Quantitative estimation of Zinc solubilization in culture medium

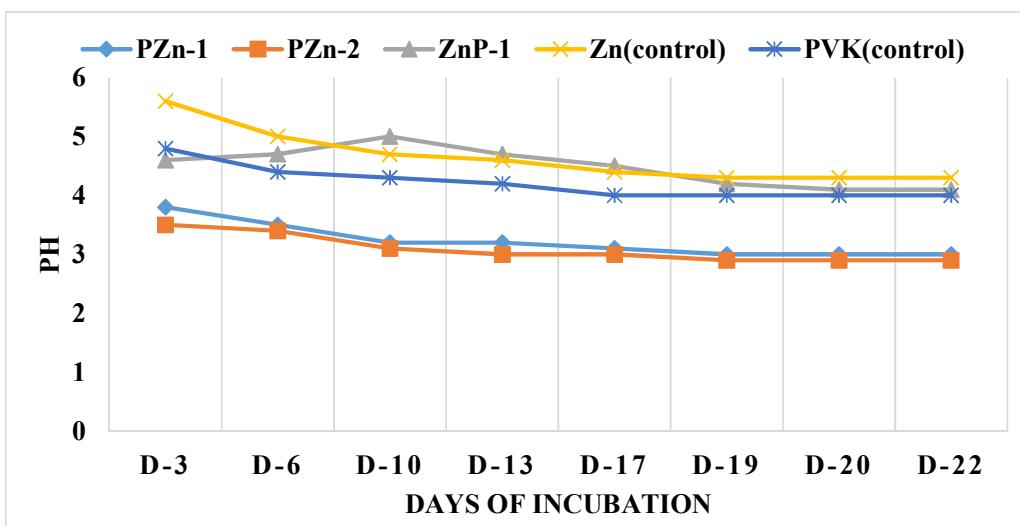
Isolates	PVK broth ($\mu\text{g/ml}$)		ZnS broth ($\mu\text{g/ml}$)	
	Day 7	Day 9	Day 10	Day 13
PZn-1	4.50	5.75	37.75	39.87
PZn-2	2.50	3.90	40.75	41.75
ZnP-1	8.25	9.50	41.00	42.25
control	1.50	2.85	13.50	14.10
SEm(\pm)	0.44	0.003	5.92	0.15
CD at 5%	1.80	0.010	NS	0.51

5) pH value of culture medium.

Data pertaining to table 4.5 and fig 4.3 depicted the pH value of isolates recorded at different days in PVK and ZnS broth as compared to control and it varied from 2.9 to 4.6. We have observed lowest pH 2.9 in PZn-2 isolate at day 19th and highest pH was observed as 4.6 in ZnP-1 isolate. The decreasing trend of pH was observed till day 17 after that pH attains stationary phase.

Table 4.5: pH of isolates in culture medium with successive days of incubation

Isolates	D-3	D-6	D-10	D-13	D-17	D-19	D-20	D-22
PZn-1	3.8	3.5	3.2	3.2	3.1	3.0	3.0	3.0
PZn-2	3.5	3.4	3.1	3.0	3.0	2.9	2.9	2.9
ZnP-1	4.6	4.7	5.0	4.7	4.5	4.2	4.1	4.1
Zn(control)	5.6	5.0	4.7	4.6	4.4	4.3	4.3	4.3
PVK(control)	4.8	4.4	4.3	4.2	4.0	4.0	4.0	4.0

**Fig 4.3: pH of isolates with successive days of incubation**

4.3 Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ on rhizospheric soil properties

4.3.1 Soil physico-chemical properties

1) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil pH at growth stage of wheat

Data referring to Table 4.6 unveiled that application of TCP, ZnCO₃ and PZn and ZnP isolates was non-significant ($P < 0.05$) and decreased the soil pH at growth stage of wheat crop. At tillering initiation, earhead initiation and maturity stage, addition of TCP and ZnCO₃ decreased the soil pH. The lowest decline viz. 7.44, 7.35, and 7.11 was observed with ZnCO₃ by 2.61%, 2.26% and 2.46% over control (P₀) respectively. Application of PZn and ZnP isolates decreased the pH in soil. The lowest decline viz. 7.46, 7.39 and 7.14 was observed in B₄ (Consortium) by 0.92%, 0.80 % and 2.32% as compared with control (B₀) at tillering initiation, earhead initiation and maturity stage respectively. The pH value ranged from 7.11 to 7.64 irrespective of treatments. Non-significant interaction was recorded between P&Zn sources and PZn & ZnP isolates at each tillering initiation, earhead initiation and maturity stage of wheat crop. Lowest soil pH was recorded in P₂B₄ (6.30%) at maturity stage as compared to control.

Table 4.6: Effect of PZn and ZnP, TCP and ZnCO₃ on soil pH at growth stage of wheat.

Treatments	Tillering stage	Initiation	Ear head initiation	Maturity stage
P and Zn source				
P ₀	7.64		7.52	7.29
P ₁ (TCP)	7.51		7.44	7.26
P ₂ (ZnCO ₃)	7.44		7.35	7.11
SEm(±)	0.06		0.07	0.07
CD at 5%	NS		NS	NS
Isolates				
B ₀ (Control)	7.53		7.45	7.31
B ₁	7.59		7.47	7.24
B ₂	7.56		7.44	7.22
B ₃	7.50		7.42	7.20
B ₄ (Consortium)	7.46		7.39	7.14
SEm(±)	0.07		0.09	0.09
CD at 5%	NS		NS	NS
F probability test				
P(TCP& ZnCO ₃)	NS		NS	NS
B(PSRB & ZnSRB)	NS		NS	NS
P*B (CD at 5%)	NS		NS	NS

*NS indicates non-significant values

2) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on organic carbon (g kg⁻¹) at growth stage of wheat.

Data referring to Table 4.7 unveiled that application of TCP, ZnCO₃ and PZn and ZnP isolates significantly ($P < 0.05$) increased the soil organic carbon at growth stage of wheat crop. At each tillering initiation, earhead initiation and maturity stage, addition of TCP and ZnCO₃ increased the soil organic carbon. The highest increase viz. 4.86 g kg⁻¹, 4.89 g kg⁻¹ and 5.01 g kg⁻¹ was observed with TCP by 12.76%, 11.36% and 12.0% over control (P₀) at each tillering initiation, earhead initiation and maturity stage respectively. Application of PZn and ZnP isolates also increased the soil organic carbon. The highest increase viz. 4.90 g kg⁻¹, 5.04 g kg⁻¹ and 5.19 g kg⁻¹ was observed in B₄ (Consortium) by 11.36%, 14.28% and 14.31% as compared with control (B₀) at each tillering initiation, earhead initiation and maturity stage. The OC value ranged from 4.31 g kg⁻¹ to 5.19 g kg⁻¹ irrespective of treatments. There was significant interaction between P&Zn sources and PZn & ZnP isolates at earhead initiation and maturity stage. Highest OC was recorded in P₁B₄ (22.49%) at tillering initiation stage, 23.42% at earhead initiation stage and 24.47% at maturity stage as compared to control.

Table 4.7: Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil organic C (g kg⁻¹) at growth stage of wheat

Treatments	Tillering Initiation stage(g kg ⁻¹)	Ear head initiation stage(g kg ⁻¹)	Maturity stage(g kg ⁻¹)
P & Zn source			
P ₀	4.31	4.38	4.47
P ₁ (TCP)	4.86	4.89	5.01
P ₂ (ZnCO ₃)	4.73	4.77	4.94
SEm(±)	0.06	0.06	0.05
CD at 5%	0.16	0.16	0.13
Isolates			
B ₀ (Control)	4.40	4.41	4.54
B ₁	4.53	4.54	4.68
B ₂	4.63	4.69	4.77
B ₃	4.71	4.73	4.87
B ₄ (Consortium)	4.90	5.04	5.19
SEm(±)	0.13	0.12	0.10
CD at 5%	0.37	0.36	0.29
F probability test			
P(TCP& ZnCO ₃)	S	S	S
B(PSRB & ZnSRB)	S	S	S
P*B (CD at 5%)	NS	0.50	0.41

*S indicates significant and NS indicates non-significant values

4.3.2 Soil available nutrients

3) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available N (mg kg⁻¹) status at growth stage of wheat

Data pertaining to Table 4.8 revealed that application of TCP, ZnCO₃ and PZn and ZnP isolates significantly ($P < 0.05$) increased the soil available nitrogen at growth stage of wheat crop. The addition of TCP and ZnCO₃ increased the soil available nitrogen. The highest increase viz. 90.60 mg kg⁻¹ and 93.59 mg kg⁻¹ was observed with TCP by 5.92% and 8.83% respectively over control (P₀) at both tillering initiation and earhead initiation stage respectively. Application of PZn and ZnP isolates increased the soil available nitrogen and highest increase viz. 91.43 mg kg⁻¹ and 92.74 mg kg⁻¹ was observed in B₄ (Consortium) by 5.55%, 4.67% as compared with control (B₀) at both tillering initiation and earhead initiation stage. Decline in the soil available nitrogen at maturity stage was observed with the addition of TCP, ZnCO₃ and PZn and ZnP isolates. The soil available nitrogen value ranged from 84.38 mg kg⁻¹ to 93.59 mg kg⁻¹ irrespective of treatments. There was significant interaction between P&Zn sources and PZn & ZnP isolates at each tillering initiation, earhead initiation and maturity stage. Highest available N was recorded in P₁B₄ (9.81%) at tillering initiation stage and 11.74% at earhead initiation stage as compared to control.

Table 4.8: Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available N (mg kg⁻¹) status at growth stage of wheat

Treatments	Tillering Initiation stage(mg kg ⁻¹)	Ear head initiation stage(mg kg ⁻¹)	Maturity stage(mg kg ⁻¹)
P& Zn source			
P ₀	85.53	85.99	84.38
P ₁ (TCP)	90.60	93.59	92.13
P ₂ (ZnCO ₃)	90.23	93.01	91.41
SEm(±)	0.34	0.19	0.24
CD at 5%	1.00	0.56	0.70
Isolates			
B ₀ (Control)	86.62	88.60	86.99
B ₁	87.38	90.25	88.54
B ₂	88.14	90.96	89.33
B ₃	88.88	91.78	89.85
B ₄ (Consortium)	91.43	92.74	91.85
SEm(±)	0.77	0.43	0.54
CD at 5%	2.23	1.24	1.56
F probability test			
P (TCP & ZnCO ₃)	S	S	S
B(PSRB & ZnSRB)	S	S	S
P*B (CD at 5%)	1.95	1.76	2.20

*S indicates significant value

4) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available P (mg kg⁻¹) status at growth stage of wheat.

Data pertaining to Table 4.9 unveiled that application of TCP, ZnCO₃ and PZn and ZnP isolates significantly ($P < 0.05$) increased the soil available phosphorus at growth stage of wheat crop. At both tillering initiation and earhead initiation stage, addition of TCP and ZnCO₃ increased the soil available phosphorus. The highest increase viz. 17.68 mg kg⁻¹ and 19.68 mg kg⁻¹ was recorded with TCP by 68.06% and 64.82% over control (P₀) respectively. Application of PZn and ZnP isolates increased the soil available phosphorus and maximum increase viz. 17.71 mg kg⁻¹ and 19.35 mg kg⁻¹ was observed in B₄ (Consortium) by 47.46% and 47.59 % as compared with control (B₀) at both tillering initiation and earhead initiation stage respectively. The soil available P value ranged from 10.52 mg kg⁻¹ to 19.68 mg kg⁻¹ irrespective of treatments. Decline in the soil available phosphorus at maturity stage was observed with the addition of TCP, ZnCO₃ and PZn and ZnP isolates. The significant interaction was observed in between P&Zn sources and PZn and ZnP isolates at tillering initiation and earhead initiation stage. Maximum available phosphorus was recorded in P₁B₄ (168%) at tillering initiation stage and 205% at earhead initiation stage as compared to control.

Table 4.9: Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available P (mg kg⁻¹) status at growth stage of wheat

Treatments	Tillering Initiation stage(mg kg ⁻¹)	Ear head initiation stage(mg kg ⁻¹)	Maturity stage(mg kg ⁻¹)
P& Zn source			
P ₀	10.52	11.94	11.34
P ₁ (TCP)	17.68	19.68	18.25
P ₂ (ZnCO ₃)	16.20	18.01	16.92
SEm(±)	0.10	0.16	0.14
CD at 5%	0.28	0.46	0.41
Isolates			
B ₀ (Control)	12.01	13.11	12.17
B ₁	13.23	15.14	14.43
B ₂	14.62	16.56	15.48
B ₃	16.45	18.59	17.37
B ₄ (Consortium)	17.71	19.35	18.06
SEm(±)	0.22	0.35	0.31
CD at 5%	0.62	1.02	0.91
F probability test			
P (TCP& ZnCO ₃)	S	S	S
B (PSRB & ZnSRB)	S	S	S
P*B (CD at 5%)	0.88	1.44	1.28

*S indicates significant value

5) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available K (mg kg⁻¹) status at growth stage of wheat

Data depicted in table 4.10 unveiled that application of TCP, ZnCO₃ and PZn and ZnP isolates significantly ($P < 0.05$) increased the soil available potassium at growth stage of wheat crop. At both tillering initiation and earhead initiation stage, addition of TCP and ZnCO₃ increased the soil available potassium. The highest increase viz. 91.48 mg kg⁻¹ and 93.66 mg kg⁻¹ respectively was observed with TCP by 16.41% and 16.60% over control (P₀). Application of PZn and ZnP isolates increased the soil available potassium and highest increase viz. 90.52 mg kg⁻¹ and 92.99 mg kg⁻¹ respectively was observed in B₄ (Consortium) by 11.05% and 12.38% as compared with control (B₀) at both tillering initiation and earhead initiation stage. The soil available K value ranged from 78.58 mg kg⁻¹ to 93.66 mg kg⁻¹ irrespective of treatments. However, treatments B₄ and B₃ were statistically at par with each other. Decline in the soil available potassium at maturity stage was observed with the addition of TCP, ZnCO₃ and PZn and ZnP isolates. There was significant interaction between P&Zn sources and PZn & ZnP isolates at each tillering initiation, earhead initiation and maturity stage. Highest available potassium was observed in P₁B₄ (30.38%) at tillering initiation stage and 32.93% at ear head initiation stage as compared to control.

Table 4.10: Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available K (mg kg⁻¹) status at growth stage of wheat.

Treatments	Tillering Initiation stage(mg kg ⁻¹)	Ear head initiation stage(mg kg ⁻¹)	Maturity stage(mg kg ⁻¹)
P& Zn source			
P ₀	78.58	80.32	79.30
P ₁ (TCP)	91.48	93.66	92.47
P ₂ (ZnCO ₃)	89.13	91.33	90.37
SEm(±)	0.21	0.12	0.23
CD at 5%	0.62	0.35	0.66
Isolates			
B ₀ (Control)	81.51	82.74	82.13
B ₁	84.33	86.76	85.84
B ₂	86.49	88.62	87.69
B ₃	89.15	91.10	90.05
B ₄ (Consortium)	90.52	92.99	91.22
SEm(±)	0.48	0.27	0.51
CD at 5%	1.38	0.79	1.47
F probability test			
P (TCP& ZnCO ₃)	S	S	S
B (PSRB & ZnSRB)	S	S	S
P*B (CD at 5%)	1.95	1.12	2.07

*S indicates significant value

6) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available Zn (mg kg⁻¹) status at growth stage of wheat stage.

Data referring to Table 4.11 unveiled that application of TCP, ZnCO₃ and PZn and ZnP isolates significantly ($P < 0.05$) increased the soil available zinc at tillering initiation and earhead initiation stage of wheat crop. At both tillering initiation and earhead initiation stage, addition of TCP and ZnCO₃ increased the soil available zinc. The highest increase viz. 1.63 mg kg⁻¹ and 1.70 mg kg⁻¹ was observed with ZnCO₃ by 48.18% and 50.44% respectively over control (P₀). Application of PZn and ZnP isolates increased the soil available zinc and highest increase viz. 1.47 mg kg⁻¹ and 1.53 mg kg⁻¹ was observed in B₄ (Consortium) by 25.64% and 25.40% respectively as compared with control (B₀) at tillering initiation and earhead initiation stage. The soil available Zn value ranged from 1.10 mg kg⁻¹ to 1.70 mg kg⁻¹ irrespective of treatments. Significant interaction was observed between P& Zn sources and PZn and ZnP isolates at tillering initiation and earhead initiation stage. Highest available zinc was observed in P₂B₄ (84.21%) at tillering initiation stage and 89.52% at earhead initiation stage as compared to control.

Table 4.11: Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available Zn (mg kg⁻¹) status at growth stage of wheat

Treatments	Tillering Initiation stage(mg kg ⁻¹)	Ear head initiation stage(mg kg ⁻¹)	Maturity stage(mg kg ⁻¹)
P& Zn source			
P ₀	1.10	1.13	1.11
P ₁ (TCP)	1.28	1.34	1.29
P ₂ (ZnCO ₃)	1.63	1.70	1.67
SEm(±)	0.01	0.01	0.32
CD at 5%	0.02	0.02	NS
Isolates			
B ₀ (Control)	1.17	1.22	1.19
B ₁	1.30	1.35	1.32
B ₂	1.35	1.39	1.36
B ₃	1.41	1.47	1.43
B ₄ (Consortium)	1.47	1.53	1.49
SEm(±)	0.02	0.02	0.41
CD at 5%	0.05	0.05	NS
F probability test			
P (TCP & ZnCO ₃)	S	S	NS
B (PSRB & ZnSRB)	S	S	NS
P*B (CD at 5%)	0.07	0.07	NS

*S indicates significant and NS indicates non-significant values

4.3.3 Exchangeable nutrients.

7) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Na (mg kg⁻¹) status at growth stage of wheat.

Data pertaining to Table 4.12 revealed that application of TCP, ZnCO₃ and PZn and ZnP isolates declined the soil exchangeable Na and was non-significant ($P<0.05$) at growth stage of wheat crop. At tillering initiation, earhead initiation and maturity stage, addition of TCP and ZnCO₃ declined the soil exchangeable Na and lowest decline viz. 103.90 mg kg⁻¹, 102.23 mg kg⁻¹ and 101.56 mg kg⁻¹ was observed with ZnCO₃ by 0.14%, 0.42% and 0.66% respectively over control (P₀). Application of PZn and ZnP isolates declined the exchangeable Na in soil and lowest decline viz. 102.74 mg kg⁻¹, 101.67 mg kg⁻¹ and 101.18 mg kg⁻¹ was observed in B₄ (Consortium) by 1.12%, 1.72% and 1.82% respectively as compared with control (B₀) at tillering initiation, earhead initiation and maturity stage. The soil exchangeable Na value ranged from 101.18 mg kg⁻¹ to 104.05 mg kg⁻¹ irrespective of treatments. Non-significant interaction was recorded between P&Zn sources and PZn and ZnP isolates at all growth stage. Lowest decline in soil exchangeable Na was recorded in P₁B₄ (0.42%) at maturity stage as compared to control.

Table 4.12: Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Na (mg kg⁻¹) status at growth stage of wheat

Treatments	Tillering Initiation stage (mg kg ⁻¹)	Ear head initiation stage (mg kg ⁻¹)	Maturity stage (mg kg ⁻¹)
P& Zn source			
P ₀	104.05	102.67	102.24
P ₁ (TCP)	103.98	102.37	102.10
P ₂ (ZnCO ₃)	103.90	102.23	101.56
SEm(±)	0.26	0.36	0.34
CD at 5%	NS	NS	NS
Isolates			
B ₀ (Control)	103.91	103.45	103.06
B ₁	104.37	104.00	103.37
B ₂	103.89	103.50	102.52
B ₃	103.34	102.74	101.83
B ₄ (Consortium)	102.74	101.67	101.18
SEm(±)	0.58	0.80	0.76
CD at 5%	NS	NS	NS
F probability test			
P (TCP& ZnCO ₃)	NS	NS	NS
B (PSRB & ZnSRB)	NS	NS	NS
P*B (CD at 5%)	NS	NS	NS

*NS indicates non-significant values

8) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Ca (me/100 g) status at growth stage of wheat.

Data referring to Table 4.13 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates significantly ($P < 0.05$) increased the soil exchangeable Ca at growth stage of wheat crop. At both tillering initiation and earhead initiation stage, addition of TCP and ZnCO₃ increased the soil exchangeable Ca. The highest increase viz. 1.79 me/100g and 1.91 me/100g was observed with TCP by 16.99% and 17.17% over control (P₀) respectively. Application of PZn and ZnP isolates increased the soil exchangeable Ca and maximum increase viz. 1.70 me/100g, 1.85 me/100g and 1.77 me/100g was observed with B₄ (Consortium) by 10.38%, 14.72% and 16.45% as compared with control (B₀) at each tillering initiation, earhead initiation and maturity stage respectively. The soil exchangeable Ca value ranged from 1.53 me/100g to 1.91 me/100g irrespective of treatments. Decline in the soil exchangeable Ca at maturity stage was observed with the addition of TCP, ZnCO₃ and PZn and ZnP isolates. Significant interaction was observed between TCP and PZn and ZnP isolates as well as ZnCO₃ and PZn and ZnP isolates at tillering initiation and ear head initiation stage. Highest exchangeable Ca was observed with P₁B₄(28.47%) at tillering initiation stage and 39.18% at earhead initiation stage as compared to control.

Table 4.13:-Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Ca (me/100g) status at growth stage of wheat.

Treatments	Tillering Initiation stage(me/100g)	Ear head initiation stage(me/100g)	Maturity stage(me/100g)
P& Zn source			
P ₀	1.53	1.63	1.58
P ₁ (TCP)	1.79	1.91	1.84
P ₂ (ZnCO ₃)	1.57	1.72	1.65
SEm(±)	0.01	0.01	0.04
CD at 5%	0.03	0.03	0.11
Isolates			
B ₀ (Control)	1.54	1.61	1.54
B ₁	1.55	1.67	1.62
B ₂	1.59	1.73	1.66
B ₃	1.62	1.73	1.70
B ₄ (Consortium)	1.70	1.85	1.77
SEm(±)	0.01	0.02	0.08
CD at 5%	0.03	0.06	NS
F Probability test			
P(TCP&ZnCO ₃)	S	S	S
B(PSRB & ZnSRB)	S	S	NS
P*B (CD at 5%)	0.05	0.09	NS

*S indicates significant and NS indicates non-significant values

9) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Mg (me/100 g) status at growth stage of wheat

Data referring to Table 4.14 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates increased the soil exchangeable Mg at growth stage of wheat crop. At both tillering initiation and earhead initiation stage, addition of TCP and ZnCO₃ significantly ($P < 0.05$) increased the soil exchangeable Mg. The highest increase viz. 1.39 me/100g and 1.49 me/100g was observed with TCP by 21.92% and 20.16% over control (P₀). Application of PZn and ZnP isolates increased the soil exchangeable Mg and was non-significant. The highest increase viz. 1.39 me/100g and 1.49 me/100g was observed in B₄ (Consortium) by 13% and 17.32 % as compared with control (B₀) at both tillering initiation and earhead initiation stage respectively. The soil exchangeable Mg value ranged from 1.14 me/100g to 1.49 me/100g irrespective of treatments. However, treatments B₄ and B₃ were statistically at par with each other. Decline in the soil exchangeable Mg at maturity stage was observed with the addition of TCP, ZnCO₃ and PZn and ZnP isolates. There was non-significant interaction between P&Zn sources and PZn & ZnP isolates at each growth stage. Maximum exchangeable Mg was observed in P₁B₄ (20.47%) at tillering initiation stage and 37.39% at earhead initiation stage.

Table 4.14: Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Mg (me/100g) status at growth stage of wheat

Treatments	Tillering Initiation stage(me/100g)	Ear head initiation stage(me/100g)	Maturity stage(me/100g)
P& Zn source			
P ₀	1.14	1.24	1.16
P ₁ (TCP)	1.39	1.49	1.43
P ₂ (ZnCO ₃)	1.34	1.41	1.38
SEm(±)	0.04	0.04	0.03
CD at 5%	0.11	0.12	0.10
Isolates			
B ₀ (Control)	1.23	1.27	1.25
B ₁	1.22	1.33	1.26
B ₂	1.25	1.35	1.28
B ₃	1.35	1.46	1.40
B ₄ (Consortium)	1.39	1.49	1.42
SEm(±)	0.09	0.09	0.08
CD at 5%	NS	NS	NS
F probability test			
P (TCP & ZnCO ₃)	S	S	S
B (PSRB & ZnSRB)	NS	NS	NS
P*B (CD at 5%)	NS	NS	NS

*S indicates significant and NS indicates non-significant values

4.3.4) Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ on biological properties.

1) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on microbial biomass carbon ($\mu\text{g}^{-1}\text{soil}$)

Data referring to Table 4.15 and unveiled that TCP, ZnCO₃ and PZn and ZnP isolates significantly ($P < 0.05$) increased the soil microbial biomass carbon at growth stage of wheat crop. At both tillering initiation and earhead initiation stage, addition of TCP and ZnCO₃ increased the soil microbial biomass carbon. The highest increase viz. 92.53 $\mu\text{g}^{-1}\text{soil}$ and 93.97 $\mu\text{g}^{-1}\text{soil}$ was observed with TCP by 6.17% and 6.9% respectively over control (P₀). Application of PZn and ZnP isolates increased the soil microbial biomass carbon and highest increase viz. 91.72 $\mu\text{g}^{-1}\text{soil}$ and 93.00 $\mu\text{g}^{-1}\text{soil}$ was observed in B₄ (Consortium) by 2.58%, 2.66% as compared with control (B₀) at both tillering initiation and earhead initiation stage respectively. The soil microbial biomass carbon value ranged from 87.15 $\mu\text{g}^{-1}\text{soil}$ to 93.97 $\mu\text{g}^{-1}\text{soil}$ irrespective of treatments. However, treatments B₄ and B₃ were statistically at par with each other. Decline in the soil microbial biomass carbon at maturity stage was observed with the addition of TCP, ZnCO₃ and PZn and ZnP isolates. There was significant interaction between P&Zn sources and PZn & ZnP isolates at tillering initiation and earhead initiation stage. Highest microbial biomass carbon was observed in P₁B₄ (8.38%) at tillering initiation stage and 10.11% at earhead initiation stage as compared to control.

Table 4.15: Effect of PZn and ZnP isolates, TCP and ZnCO₃ on microbial biomass carbon ($\mu\text{g}^{-1}\text{soil}$) status at growth stage of wheat

Treatments	Tillering Initiation stage ($\mu\text{g}^{-1}\text{soil}$)	Ear head initiation stage ($\mu\text{g}^{-1}\text{soil}$)	Maturity stage ($\mu\text{g}^{-1}\text{soil}$)
P and Zn source			
P ₀	87.15	87.90	87.53
P ₁ (TCP)	92.53	93.97	93.33
P ₂ (ZnCO ₃)	92.36	93.77	93.17
SEM(\pm)	0.05	0.07	0.04
CD at 5%	0.14	0.20	0.11
Isolates			
B ₀ (Control)	89.41	90.59	89.63
B ₁	90.13	91.42	90.91
B ₂	90.75	91.97	91.43
B ₃	91.40	92.46	91.97
B ₄ (Consortium)	91.72	93.00	92.81
SEM(\pm)	0.11	0.16	0.09
CD at 5%	0.32	0.45	0.25
F probability test			
P (TCP & ZnCO ₃)	S	S	S
B (PSRB & ZnSRB)	S	S	S
P*B (CD at 5%)	0.46	0.64	0.36

*S indicates significant value

2) Effect of PZn and ZnP, TCP and ZnCO₃ on soil dehydrogenase activity ($\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$).

Data referring to Table 4.16 and unveiled that TCP, ZnCO₃ and PZn and ZnP isolates significantly ($P < 0.05$) increased the soil dehydrogenase activity at growth stage of wheat crop. At both tillering initiation and earhead initiation stage, addition of TCP and ZnCO₃ increased the soil dehydrogenase activity. The highest increase viz. 57.90 $\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$ and 59.10 $\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$ was observed with TCP by 20.97% and 22.18% over control (P₀). Application of PZn and ZnP isolates increased the soil dehydrogenase activity and highest increase viz. 57.82 $\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$ and 58.61 $\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$ was observed in B₄ (Consortium) by 14.04% and 13.23% as compared with control (B₀) at both tillering initiation and earhead initiation stage respectively. The soil dehydrogenase activity value ranged from 47.86 $\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$ to 59.10 $\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$ irrespective of treatments. Decline in the soil dehydrogenase activity at maturity stage was observed with the addition of TCP, ZnCO₃ and PZn and ZnP isolates. There was significant interaction between P& Zn sources

and PZn and ZnP isolates at tillering initiation and earhead initiation stage. Highest dehydrogenase activity was recorded in P₁B₄ (33.92%) at tillering initiation stage and 37.03% at earhead initiation stage as compared to control respectively.

Table 4.16: Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil dehydrogenase activity ($\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$) at growth stage of wheat

Treatments	Tillering initiation stage ($\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$)	Ear head initiation stage ($\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$)	Maturity stage ($\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$)
P& Zn source			
P₀	47.86	48.37	48.21
P₁(TCP)	57.90	59.10	58.67
P₂ (ZnCO₃)	55.84	56.18	56.05
SEm(±)	0.08	0.17	0.09
CD at 5%	0.22	0.48	0.24
Isolates			
B₀ (Control)	50.70	51.76	51.14
B₁	52.72	53.15	53.09
B₂	53.34	54.14	54.02
B₃	54.79	56.16	55.66
B₄(Consortium)	57.82	58.61	58.26
SEm(±)	0.17	0.37	0.13
CD at 5%	0.49	1.07	0.35
F probability test			
P(TCP& ZnCO₃)	S	S	S
B(PSRB&ZnSRB)	S	S	S
P*B (CD at 5%)	0.69	1.52	0.47

*S indicates significant value

3) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil phosphatase activity

($\mu\text{g-PNP g}^{-1}\text{soil hr}^{-1}$).

Data referring to Table 4.17 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates significantly ($P < 0.05$) increased the soil phosphatase activity at growth stage of wheat crop. At both tillering initiation and earhead initiation stage, addition of TCP and ZnCO₃ increased the soil phosphatase activity. The highest increase viz. 48.09 $\mu\text{g-PNP g}^{-1}\text{soil hr}^{-1}$ and 49.06 $\mu\text{g-PNP g}^{-1}\text{soil hr}^{-1}$ was observed with TCP by 28.30% and 26.86% over control (P₀). Application of PZn and ZnP isolates increased the soil phosphatase activity and highest increase viz. 47.95 $\mu\text{g-PNP g}^{-1}\text{soil hr}^{-1}$ and 48.82 $\mu\text{g-PNP g}^{-1}\text{soil hr}^{-1}$.

PNP g⁻¹soil hr⁻¹ was observed in B₄ (Consortium) by 20.56%, and 19.53 % as compared with control (B₀) at both tillering initiation and earhead initiation stage. The soil phosphatase activity value ranged from 37.48 µg-PNP g⁻¹soil hr⁻¹ to 49.06 µg-PNP g⁻¹soil hr⁻¹ irrespective of treatments. Decline in the soil phosphatase activity at maturity stage was observed with the addition of TCP, ZnCO₃ and PZn and ZnP isolates. There was significant interaction between P& Zn sources and PZn and ZnP isolates at tillering initiation and earhead initiation stage. Highest phosphatase activity was observed in P₁B₄ 55.74% at tillering initiation stage and 55.53% at earhead initiation stage over control.

Table 4.17: Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil phosphatase activity (µg- PNP g⁻¹soil hr⁻¹) at growth stage of wheat

Treatments	Tillering Initiation stage (µg-PNP g ⁻¹ soil hr ⁻¹)	Ear head initiation stage (µg- PNP g ⁻¹ soil hr ⁻¹)	Maturity stage (µg-PNP g ⁻¹ soil hr ⁻¹)
P& Zn source			
P ₀	37.48	38.67	37.90
P ₁ (TCP)	48.09	49.06	48.62
P ₂ (ZnCO ₃)	46.18	47.21	46.89
SEm(±)	0.08	0.05	0.91
CD at 5%	0.23	0.14	NS
Isolates			
B ₀ (Control)	39.77	40.84	40.10
B ₁	42.48	43.52	42.27
B ₂	43.32	44.50	43.54
B ₃	46.09	47.25	46.70
B ₄ (Consortium)	47.95	48.82	48.02
SEm(±)	0.18	0.11	1.04
CD at 5%	0.51	0.32	2.91
F probability test			
P(TCP& ZnCO ₃)	S	S	NS
B(PSRB& ZnSRB)	S	S	S
P*B (CD at 5%)	0.73	0.45	0.46

*S indicates significant and NS indicates non-significant values

4.4 Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ isolates on plant properties of wheat crop.

1) Nutrient uptake (N, P, K & Zn) (g pot⁻¹).

Data referring to Table 4.18 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates significantly (P< 0.05) increased the soil nutrient uptake in wheat crop.

Addition of TCP and ZnCO₃ increased N, P, K & Zn. The highest increase viz. N (20.11g pot⁻¹ in grain, 13.68 g pot⁻¹ in straw), P(4.58 g pot⁻¹ in grain, 4.30 g pot⁻¹ in straw) and K(4.87 g pot⁻¹ in grain, 27.28 g pot⁻¹ in straw) was observed with TCP and increase was estimated in N by 46.25% in grain and 63.83% in straw, P by 104.46 % in grain and 118.27% in straw and K by 124.4% in grain and 23.8% in straw in case of Zn uptake highest increase was observed with ZnCO₃ viz. 27.25 g pot⁻¹ in grain, 64.74 g pot⁻¹ in straw by 13.87% in grain, and 7.45% in straw over control P₀ respectively. Application of PZn and ZnP isolates increased N, P, K & Zn uptake in wheat crop and maximum increase viz. N (19.40 g pot⁻¹ in grain, 13.43 g pot⁻¹ in straw), P(4.73 g pot⁻¹ in grain, 4.45 g pot⁻¹ in straw), K(4.51 g pot⁻¹ in grain, 27.93 g pot⁻¹ in straw) and Zn(27.05 g pot⁻¹ in grain, 64.89 g pot⁻¹ in straw) was observed with B₄ in N by 29.67% in grain and 47.74% in straw, P by 74.53 % in grain and 83.12% in straw, K by 78.26% in grain and 20.28% in straw and Zn by 10.99% in grain and 6.86% in straw as compared with control (B₀) respectively. There was significant interaction between P&Zn sources and PZn and ZnP isolates for the nutrient uptake in wheat crop. Maximum increase in nutrient uptake was observed in P₁B₄ N uptake (94.36% in grain & 144.89% in straw), P uptake (452% in grain & 615.38% in straw), K uptake (273% in grain & 54.25% in straw) and in case of Zn uptake remarkable increase was observed with P₂B₄ by 18.86% in grain and 14.48% in straw over control.

Table 4.18:-Effect of PZn and ZnP isolates, TCP and ZnCO₃ on N, P, K & Zn uptake at harvest (g pot⁻¹).

Treatments	N-Uptake (g pot ⁻¹)		P-Uptake (g pot ⁻¹)		K-Uptake (g pot ⁻¹)		Zn-Uptake (g pot ⁻¹)	
P& Zn source	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
P ₀	13.75	8.35	2.24	1.97	2.17	22.02	23.93	60.25
P ₁ (TCP)	20.11	13.68	4.58	4.30	4.87	27.28	26.17	63.43
P ₂ (ZnCO ₃)	17.73	11.54	4.13	3.85	3.71	26.63	27.25	64.74
SEm(±)	0.09	0.12	0.05	0.04	0.05	0.11	0.11	0.19
CD at 5%	0.27	0.36	0.13	0.12	0.16	0.32	0.32	0.54
Isolates								
B ₀ (Control)	14.96	9.09	2.71	2.43	2.53	23.22	24.37	60.72
B ₁	16.49	10.32	3.01	2.74	3.35	24.12	25.16	62.10
B ₂	17.06	11.13	3.67	3.39	3.51	25.09	25.32	62.28
B ₃	18.10	12.01	4.16	3.88	4.05	26.22	27.05	64.05
B ₄ (Consortium)	19.40	13.43	4.73	4.45	4.51	27.93	27.05	64.89
SEm(±)	0.21	0.28	0.10	0.09	0.12	0.25	0.24	0.41
CD at 5%	0.60	0.80	0.30	0.26	0.35	0.71	0.70	1.20
F probability test								
P(TCP&ZnCO ₃)	S	S	S	S	S	S	S	S
B(PSRB&ZnSRB)	S	S	S	S	S	S	S	S
P*B (CD at 5%)	0.86	1.14	0.46	0.37	0.49	1.01	1.00	1.69

*S indicates significant value

2) Yield attributes

2.1 Effect of PZn and ZnP isolates, TCP and ZnCO₃ on plant height (cm), average ear length (cm), no. of grains earhead⁻¹, grain yield and straw yield (g pot⁻¹)

Data referring to Table 4.19 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates significantly (P< 0.05) increased the plant height, average ear length, no. of grains earhead⁻¹, grain yield and straw yield in wheat crop. Addition of TCP and ZnCO₃ increased yield attributes. The maximum increase viz. 92.70 cm, 13.89 cm, 36.32, 18.56 g pot⁻¹ and 23.33 g pot⁻¹ was recorded with TCP by 22.18% in plant height, average ear length by 20.99%, no. of grains earhead⁻¹ by 8.61%, grain yield by 35.67% and straw yield by 35.79% over control P₀. Application of PZn and ZnP isolates increased plant height, average ear length, no. of grains earhead⁻¹, grain yield and straw yield in wheat crop and remarkable increase viz. 88.05 cm, 13.08cm, 35.71, 17.32 g pot⁻¹ and 21.78 g pot⁻¹ was observed with B₄ by 13.11%, 9.54%, 6.62%, 23.27% and 22.35% respectively as compared with control (B₀). Significant interaction was observed between TCP and

PZn and ZnP isolates for the plant height, average ear length, no. of grains earhead⁻¹, grain yield and straw yield in wheat crop. The highest increase in plant height, average ear length, no. of grains earhead⁻¹, grain yield and straw yield was observed with P₁B₄ viz. 37.89% in plant height, 31.71 in average ear length, 15.36% no. of grains earhead⁻¹, 66.17% in grain yield and 66.18% in straw yield as compared to control.

Table 4.19: Effect of PZn and ZnP isolates, TCP and ZnCO₃ on yield attributes of wheat at harvest

Treatments	Plant Height (cm)	Ear length (cm)	No. of grains earhead ⁻¹	Grain Yield (g pot ⁻¹)	Straw Yield (g pot ⁻¹)
P& Zn source					
P₀	75.87	11.48	33.44	13.68	17.18
P₁(TCP)	92.70	13.89	36.32	18.56	23.33
P₂ (ZnCO₃)	81.70	12.37	34.32	15.47	19.45
SEm(±)	0.18	0.07	0.01	0.09	0.12
CD at 5%	0.51	0.19	0.04	0.26	0.36
Isolates					
B₀(Control)	77.84	11.94	33.49	14.05	17.80
B₁	81.62	12.37	34.41	15.54	19.44
B₂	83.56	12.63	34.81	15.99	20.14
B₃	86.07	12.91	35.07	16.65	20.81
B₄(Consortium)	88.05	13.08	35.71	17.32	21.78
SEm(±)	0.40	0.15	0.03	0.20	0.28
CD at 5%	1.15	0.43	0.09	0.58	0.80
F probability test					
P (TCP & ZnCO₃)	S	S	S	S	S
B (PSRB & ZnSRB)	S	S	S	S	S
P*B (CD at 5%)	1.63	0.61	0.13	0.82	1.14

*S indicates significant value

4.5 Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ on Relative Efficiency of Phosphorus Use (REP %) and Relative Efficiency of Zinc Use (REZn %)

Data referring to Table 4.20 unveiled that relative efficiency of phosphorus use and relative efficiency of zinc use increased with addition of TCP, ZnCO₃ and PZn and ZnP isolates. Application of TCP and ZnCO₃ increased REP% by 74.35% and REZn% by 75.88 % over control. Addition of PZn and ZnP isolates increased REP% and REZn% and highest REP% and REZn% was observed in the treatment B₄ (consortium) by 88.39 % and 90.44% respectively as compared with control (B₀).

Table 4.20: Effect of PZn and ZnP isolates, TCP and ZnCO₃ on Relative Efficiency of Phosphorus Use (REP %) and Relative Efficiency of Zinc Use (REZn %) of wheat crop

Treatments	Dry matter (g/plant)		REP%	Dry matter (g/plant)		REZn%
	Low Pi	High Pi		Low Zn	High Zn	
P₀	0.37	0.78	47.44	0.39	0.80	48.75
P₁ (TCP)	1.16	1.56	74.35	1.20	1.63	73.61
P₂ (ZnCO₃)	1.09	1.51	72.18	1.29	1.70	75.88
B₀ (Control)	1.13	1.54	73.37	1.22	1.64	74.39
B₁ (PZn- 1)	2.58	3.19	80.88	2.65	3.23	82.04
B₂ (PZn- 2)	3.32	4.05	81.98	3.49	4.21	82.89
B₃ (ZnP-1)	3.45	4.20	82.14	3.57	4.29	83.21
B₄ (Consortium)	4.11	4.65	88.39	4.26	4.71	90.44

Discussion

Chapter 5

Discussion

On the basis of outcomes achieved throughout the research study an effort has been made to pronounce the causes of disparities obtained owing to TCP as phosphorus source, $ZnCO_3$ as zinc source and use of PZn and ZnP isolates. The outcomes have been discussed with the literature existing on the diverse parameters underneath the study with subsequent heads:

5.1 Isolation and Biochemical identification of isolates (P&Zn isolates).

- 1) Isolation of P & Zn Solubilizing rhizobacteria
- 2) Biochemical identification of isolates

5.2 Mechanisms of Phosphate and Zinc Solubilization by PZn and ZnP isolates in culture medium

- 1) Qualitative estimation of phosphate solubilization
- 2) Qualitative estimation of zinc solubilization
- 3) Quantitative estimation of phosphate solubilization
- 4) Quantitative estimation of zinc solubilization

5.3 Inoculation of PZn and ZnP isolates along with TCP and $ZnCO_3$ on rhizospheric soil properties

5.3.1 Soil physico-chemical properties.

- 1) Effect of PZn and ZnP isolates, TCP and $ZnCO_3$ on soil pH at growth stage of wheat
- 2) Effect of PZn and ZnP isolates, TCP and $ZnCO_3$ on organic carbon ($mg\ kg^{-1}$) at growth stage of wheat

5.3.2 Soil available nutrients.

- 3) Effect of PZn and ZnP isolates, TCP and $ZnCO_3$ on soil available N ($mg\ kg^{-1}$) status at growth stage of wheat
- 4) Effect of PZn and ZnP isolates, TCP and $ZnCO_3$ on soil available P ($mg\ kg^{-1}$) status at growth stage of wheat

- 5) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available K (mg kg⁻¹) status at growth stage of wheat
- 6) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available Zn (mg kg⁻¹) status at growth stage of wheat

5.3.3 Exchangeable nutrients.

- 7) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Na (mg kg⁻¹) status at growth stage of wheat
- 8) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Ca (me/100 g) status at growth stage of wheat
- 9) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Mg (me/100 g) status at growth stage of wheat

5.3.4 Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ on biological properties

- 1) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil microbial biomass carbon (μg^{-1} soil)
- 2) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil dehydrogenase activity ($\mu\text{g-TPF g}^{-1}\text{soil hr}^{-1}$)
- 3) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil phosphatase activity ($\mu\text{g- PNP g}^{-1}\text{soil hr}^{-1}$)

5.4 Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ isolates on plant properties of wheat crop

1) Nutrient uptake (N, P, K & Zn) (g/pot)

2) Yield attributes

- 2.1 Plant height (cm)
- 2.2 Average ear length (cm)
- 2.3 No. of grains earhead⁻¹
- 2.4 Grain and straw Yield (g pot⁻¹)

5.5 Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ on Relative Efficiency of Phosphorus Use (REP %) and Relative Efficiency of Zinc Use (REZn %)

Biochemical identification of isolates (P&Zn isolates)

1) Biochemical identification of isolates.

The bacterial isolates isolated from rhizosphere soil of wheat crop from different locations were subjected for biochemical identification viz, Citrate utilization, Urease, Nitrate reduction and H₂S production (table 4.1). Bacteria showing positive citrate utilization reveals that such bacteria have the potential to transform salts of organic acids to organic carbon, resultantly pH raises and in the end there will be no acid (Park *et al.* 2005). Bacteria showing positive urease test reveals that they are capable of hydrolyzing urea release to NH₃ and CO₂. Positive nitrate reduction test confirms that isolates could reduce nitrate to nitrite but not to N₂ gas, this test also reveals that such isolates have potential to breakdown nitrate even in anaerobic conditions in soil (Karpagam and Nagalaxmi 2014). The bacterial strain showing positive results for H₂S production are capable of reducing sulphur compounds. The results shown in table 4.1 is in conformity with Srivastava 2013, Panhwar *et al.* 2012, Gupta *et al.* 2018 and Al Ali *et al.* 2021.

Mechanisms of Phosphate and Zinc Solubilization by PZn and ZnP isolates in culture medium

1) Qualitative estimation of phosphate solubilization.

Data referring to table 4.2 and plate 4.2 depicted the halozone development around the bacterial colonies which was attributed to phosphate solubilization in its locality and was superficial more with PZn-2 isolate. It was also observed that with increase in the incubation time, the zone size of each isolate was increased. Similar results was detected by Lavakush *et al.* (2012) by using Pikovskaya's media. These results are also in accordance with those of Cavite *et al.* 2018, Tripti and Anshumali 2012 who also described zone of formation could be due to activity of phosphates enzyme by PZn-1 isolate. High PSI index (2.82) recorded by PZn-2 isolate indicated that this strain isolated from wheat rhizosphere is an effective phosphate solubilizer which was in accordance with Chakravarthy *et al.* (2010), Paul and Sinha, 2017, Aarab *et al.* 2019 and Gupta *et al.* 2022.

2) Qualitative estimation of zinc solubilization.

Data referring to table 4.2 unveiled that increasing in the incubation time, increases the halo zone size of each isolate in respective ZnS agar. Highest SI and SE (%) was observed for ZnP-1 isolate and lowest was observed in the plate having PZn-1 isolate. The remarkable SI and SE (%) with ZnP-1 isolate might be correlated with the release of IAA and gluconic acid (Potshangbam *et al.* 2018). The demonstrated variation in the ability of solubilization by application of zinc source could be due to metabolic activity of a given strain which is in agreement with observations of Shahab and Ahmed (2008). Similar findings was reported by Bhojiya and Joshi, 2012, Jagana *et al.* 2019 and Rehman *et al.* 2021.

3) Quantitative estimation of phosphate solubilization in culture broth.

Data presented in table 4.3 and Fig 4.1 & 4.2 reported the quantitative estimation of phosphate solubilization in liquid medium (PVK broth and ZnS broth) unveiled phosphate solubilization activity in the form of extreme P discharge in culture supernatant at different days interval. Highest P solubilization was observed in broth containing PZn-2 isolate at 13th day, which could be due to different means of phosphate solubilization by all three strains after that solubilization starts declining accordingly which is totally linked with lowest pH decline at 13th day as given in the table 4.3. Similar results reported by Yadav *et al.* 2016 and Eramma *et al.* 2020 for various microorganisms with the maximum phosphate solubilization by the consortium that might be attributed to synergistic effect by PZn and ZnP isolates ultimately leads to remarkable P solubilization.

4) Quantitative estimation of zinc solubilization in broth.

Data referring to table 4.4 reported the quantitative estimation of zinc solubilization in liquid medium (PVK broth and ZnS broth) unveiled zinc solubilization activity. A significant difference was observed as compared to control at 7th and 9th day respectively in PVK broth and at 10th and 13th day respectively in ZnS broth. Incited enzyme action signified microbial growth and decline in rhizospheric pH is accountable for maximum Zn availability at 9th day in PVK broth and at 13th day in ZnS broth. The zinc solubilization in our study could also be due to production of organic acids, like gluconic acids that is augmented by the fall in pH of culture media in all isolates.

Similar findings was observed by Shakeel *et al.* 2015, Potshangbam *et al.* 2018 and Jagana *et al.* 2019.

5) Change in pH value of culture medium.

Data referring to table 4.5 and fig 4.3 reported the pH of isolates that was recorded at different days in PVK and ZnS broth against control media. It was observed that the pH in culture medium decreases with increasing in the incubation time. This might be due to the secretion of organic acids like gluconic acid, oxalic acid, citric acid, as well as other chelating metabolites (Agnihorti, 1970) of microbial origin by the isolates in their respective media as evidenced by a fall in pH of the culture medium and would also help in solubilizing phytates in soil. Similar findings was observed by Khande *et al.* 2017, Jagana *et al.* 2019 and Gupta *et al.* 2021.

5.3 Inoculation of PZn and ZnP along with TCP and ZnCO₃ on rhizospheric soil properties

5.3.1 Soil physico-chemical properties

1) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil pH at growth stage of wheat

Data referring to table 4.6 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates decreased the soil pH at growth stage of wheat crop and was non-significant. At tillering initiation, earhead initiation and maturity stage, addition of TCP and ZnCO₃ declined the soil pH. The lowest decline was observed with ZnCO₃ which might be due to the fact that the production of organic acids, i.e. formic, acetic, citric, gluconic, 2-ketogluconic, malic, lactic and oxalic acids in soils inoculated with PZn and ZnP isolates is a vital mechanism to solubilize the complex Zn into soluble form by dropping the pH of microbial vicinity (Zaheer *et al.* 2019). Our results was also in conformity with Kushwaha *et al.* 2021.

2) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on organic carbon (g kg⁻¹) at growth stage of wheat.

Data referring to table 4.7 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates significantly ($P < 0.05$) increased the soil organic carbon at growth stage of wheat crop. Highest soil organic carbon was recorded with TCP amended soil. This might be due to TCP application which promotes the crop growth including root proliferation and

this root biomass is added as organic matter to the soil. Additionally, using both organic and inorganic fertiliser sources simultaneously is a wonderful way to increase root biomass and increase crop output (Gunjal and Chitodkar 2017). Similar findings were made by Banerjee *et al.* (2011), Solanki *et al.* (2015), and Ramalakshmi *et al.* (2008). Soil inoculated with isolates recorded highest increase in soil organic carbon with B₄ (consortium) amended soil as compared to control (B₀). This might be due to multiplication of the PZn and ZnP isolates in the soil which releases a variety of substances that encourage plant growth. These substances encourage the rhizodeposition of photosynthetic carbon in the form of root exudates and they also increase overall crop growth including root biomass, which eventually decomposes into organic matter in the soil.

5.3.2 Soil available nutrients.

3) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available N (mg kg⁻¹) status at growth stage of wheat.

Data referring to table 4.8 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates amended soil observed significant ($P < 0.05$) increase in available nitrogen in soil at growth stage of crop. The maximum increase in available N in soil was observed in (P₁) TCP amended soil as compared to control owing to better root proliferation in presence of TCP and at harvest stage decrease in the availability of available N was due to surge in absorption of nitrogen by the plants. Isolates amended soil exhibited increased available nitrogen in soil at growth stage of crop and maximum surge in available nitrogen in soil was found in B₄(consortium) as compared to control due to increased N fixation by the isolates at growth stage. Alike outcomes were observed by Gulati *et al.* 2010, Saharan and Nehra 2011 and Kumar *et al.* 2021. With application of PGPR increase in rhizosphere soil nitrogen content was also recorded by Cakmakci *et al.* (2007). Decline in the accessibility of available N was attributed to increase in the absorption of nitrogen by the plants at harvest stage.

4) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil available P (mg kg⁻¹) status at growth stage of wheat.

Data referring to table 4.9 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates amended soil observed increase in available phosphorus in soil at growth stage of crop and maximum increase in available P in soil was observed in (P₁) TCP amended soil as

compared to control and ZnCO_3 , which was in accord with Singh *et al.* 2014. Decline in the available phosphorus in soil might be owing to surged absorption of phosphorus by plants during their growth at harvest stage. PZn and ZnP isolates amended soil displayed increased available phosphorus content in soil at growth stage of crop and maximum increase in available phosphorus in soil was observed in B₄ (consortium) as compared to control due to increased phosphate solubilization activity of both the microorganisms that might have released P from unavailable form to available form by the production of organic acids and phosphates enzymes which was in consensus with Sundara *et al.* 2002 and Kumar *et al.* 2021. Remarkable decline in the available phosphorus in soil was attributed to surged absorption of phosphorus by plants during their growth at harvest stage.

5) Effect of PZn and ZnP isolates, TCP and ZnCO_3 on soil available K (mg kg^{-1}) status at growth stage of wheat.

Data referring to table 4.10 unveiled that TCP, ZnCO_3 and PZn and ZnP isolates amended soil observed increase in available potassium in soil at growth stage of crop and maximum upsurge in available K in soil was observed in (P₁) TCP amended soil as compared to control and ZnCO_3 which was in accord with Singh *et al.* 2014. Decline in the available potassium in soil might be owing to surged absorption of potassium by plants during their growth at harvest stage. Isolates amended soil revealed the increase in the available potassium in soil at growth stage of crop and maximum increase in available potassium in soil was observed in B₄ (consortium) as compared to control due to production of organic acids by the isolates which aid in discharge of mineral bond insoluble potassium that might have declined potassium fixation which was in accord with Vyas *et al.* 2009. Decline in the available potassium in soil was attributed to surged absorption of potassium by plants during their growth at harvest stage.

6) Effect of PZn and ZnP isolates, TCP and ZnCO_3 on soil available Zn (mg kg^{-1}) status at growth stage of wheat.

Data referring to table 4.11 unveiled that TCP, ZnCO_3 and PZn and ZnP isolates amended soil observed increase in available zinc in soil at growth stage of crop and maximum increase in available Zn in soil was observed in (P₂) ZnCO_3 amended soil as compared to control, which was also reported by Kushwaha *et al.* 2021. Isolates amended soil shows the increase in the available zinc in soil at growth stage of crop

and maximum increase in available zinc in soil was observed in B₄ (consortium) as equated to control. This might be attributed by the production of organic acids i.e., malic, lactic, formic, acetic, citric, gluconic, 2-ketogluconic, and oxalic acids in soils inoculated with ZnSB is a key mechanism to solubilize the complex Zn into soluble form by lowering the pH of microbial surrounding (Zaheer *et al.* 2019), thus increasing Zn availability and assimilation in plants.

5.3.3 Exchangeable nutrients

7) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Na (mg kg⁻¹) status at growth stage of wheat.

Data referring to table 4.12 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates amended soil observed decline in exchangeable sodium in soil at growth stage of crop and lowest decline in exchangeable sodium in soil was observed in (P₁) TCP amended soil as compared to control. Isolates amended soil exhibited decline in the exchangeable sodium at growth stage of crop and lowest decline was observed in B₄ (consortium) as compared to control due to low CEC sodium is held less strongly by the soil particles. Alike, results were stated by Hafez *et al.* 2019.

8) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Ca (me/100 g) status at growth stage of wheat.

Data referring to table 4.13 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates amended soil observed increase in the exchangeable Ca content at maturity stage that might be due to increased Ca ions concentrations in soil solution by retaining Na ions onto exchange sites and releasing other nutrients into the soil solution Hafez *et al.* 2019. Isolates amended soil revealed increase in exchangeable Ca at growth stage of wheat crop due to increased Ca ions concentrations in soil solution by deploying Na ions onto exchange sites and releasing other nutrients into the soil solution Hafez *et al.* 2019. Similar results were reported by Chungopast *et al.* 2021.

9) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil exchangeable Mg (me/100 g) status at growth stage of wheat.

Data referring to table 4.14 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates amended soil observed significant increase in exchangeable Mg content at both stage owing to release of acids, a lot of acid cations are held by the soil particles and increase in cation exchange capacity of soils as compared with control. Because of the low pH,

acidification, and solubilization of insoluble cations in isolated treated soil, wheat crop growth stage exhibited a non-significant increase in exchangeable Mg. The soil particles hold a lot of acid cations, and soils have a higher cation exchange capacity. Sindhu *et al.* (2019) reported findings that were comparable. From the vegetative stage to the maturity stage, it was observed that the exchangeable magnesium content of soil decreased gradually. This decrease might have been attributed by crop removal and other soil changes (Lim *et al.* 2020)

5.3.4 Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ on biological properties

1) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil microbial biomass carbon (μg^{-1} soil)

Data referring to table 4.15 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates amended soil observed increase in soil microbial biomass carbon and maximum increase was recorded in treatment P₁ (TCP) as compared to control and ZnCO₃ due to the organic substances released from the roots to the rhizosphere soil supporting higher microbial biomass in the rhizosphere as well as due to supply of mineralization of C and N resulting in enhancement of indigenous microflora which was in accordance with Raghubeer *et al.* 2017 and showed decrease at harvest stage revealing no activity of microbes at this stage. Isolates amended soil showed increase in soil microbial biomass carbon and highest increase was recorded in B₄(consortium) as compared to control due to the organic substances released from the roots to the rhizosphere soil supporting higher microbial biomass in the rhizosphere as well as due to supply of mineralization of C and N resulting in enhancement of indigenous microflora. At harvest stage decrease in the soil microbial biomass carbon revealed low activity of microbes.

2) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil dehydrogenase activity ($\mu\text{g-TPFg}^{-1}\text{soil hr}^{-1}$).

Data referring to table 4.16 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates amended soil showed remarkable increase in dehydrogenase activity and highest increase was recorded in treatment P₁ (TCP) as compared to control due to greater microbial and root activity in the rhizosphere which was in accordance with Stephen *et al.* 2015. Isolates amended soil recorded increase in the dehydrogenase activity of soil

and highest increase was observed in treatment B₄ (consortium) as compared to control due to greater microbial and root activity in the rhizosphere which was in accordance with Raghuveer *et al.* 2017 and with a decrease in the dehydrogenase activity at harvest stage due to decreased microbial activity in the rhizosphere.

3) Effect of PZn and ZnP isolates, TCP and ZnCO₃ on soil phosphatase activity ($\mu\text{g- PNP g}^{-1}\text{soil hr}^{-1}$).

Data referring to table 4.17 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates amended soil observed increase in phosphatase activity and maximum increase was observed in treatment P₁ (TCP) due to enriched accessibility of P contents in the plant rhizosphere (Madhaiyan *et al.* 2010; Gopalakrishnan *et al.* 2016) and decline in phosphatase activity was observed in harvest stage. Isolates amended soil recorded increase in phosphatase activity of soil and highest increase was observed in B₄ (consortium) as compared to control due to increase in P release as this enzyme has its activity more during P deficiency which was in accordance with Sreelakshmi *et al.* 2019.

5.4.4 Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ on plant properties of wheat crop

1) Nutrient uptake (N, P, K & Zn) (g pot^{-1}).

Data referring to table 4.18 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates soil recorded increase in N, P, K uptake in wheat crop with maximum increase in P₁ treatment (TCP) and B₄ (consortium) which was in consensus with Viruel *et al.* 2014 and Lin *et al.* 2016. Kuan *et al.* 2016 also revealed that the microbially induced plant growth regulators like auxin stimulate lateral and adventitious root establishment, which boosts general plant nutrient uptake. Similar results was also reported by Afzal and Bano 2008. But in case of Zn uptake, maximum increase was recorded in P₂ (ZnCO₃) this might be due to stimulation of gene regulators like Zur causative in Zn uptake and mobilization in plants (Mikhaylina *et al.* 2018; Kandari *et al.* 2019). In isolates amended soil highest nutrient uptake was observed with treatment B₄ (consortium) as compared to control in grain and straw that might be the result of the treatment combination of bacterial strains that are much better adapted to the soil, which has a synergistic and additive impact that greatly facilitates the uptake of crucial nutrients by crops from soils (Kumar *et al.* 2021).

2) Yield attributes.

2.1 Effect of PZn and ZnP isolates, TCP and ZnCO₃ on plant height (cm), average ear length (cm), no. of grains earhead⁻¹, grain yield and straw yield (g/pot)

Data referring to table 4.19 unveiled that TCP, ZnCO₃ and PZn and ZnP isolates amended soil recorded increase in yield and yield attributes with maximum increase in TCP amended soil and B₄(consortium) due to better supply of phosphorus, prolific root growth resulting in enhanced water and nutrient absorption. It is attributed to the improvement of the soil environment which encouraged proliferation of roots resulting in more absorption of nutrients and water from larger area and depth as also reported by Lavakush *et al.* 2014, Deshwal and Kumar 2013 and Hameeda *et al.* 2006. The similar results were also reported by Hossain and Sattar (2014) and Afzal and Bano 2008. Sharma *et al.* (2012) exhibited that the blend of PSB and phosphorus has enormous contribution in cell division, root elongation and commanding constituent of ATP and ADP which are accountable for the yield and yield component increment.

5.5 Inoculation of PZn and ZnP isolates along with TCP and ZnCO₃ on Relative Efficiency of Phosphorus Use (REP %) and Relative Efficiency of Zinc Use (REZn %)

Data referring to this effect in table 4.20 unveiled that relative efficiency of phosphorus increase with the highest REP% found in consortium that might be due to the increase in P release and increase in P solubilization capacity of the isolates which was in accordance with Kshetri *et al.* 2018. Relative efficiency of zinc increase and highest REZn% was found in consortium which might be attributed to synergistic relationship between the PZn and ZnP isolates and consortium. Combined inoculation of PZn and ZnP isolates showed increase in Zn solubilization and Zn uptake, ultimately leads to enhancement in REZn% which was in conformity with Akhtar *et al.* 2013; Sajjad *et al.* 2001; De Freitas 2000.

Summary and Conclusion

Chapter 6

Summary and Conclusions

The present study entitled “Isolation, Identification and Evaluation of phosphate and zinc Solubilizers in wheat rhizosphere” was carried out at Division of Soil Science and Agricultural Chemistry of Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu during 2021-2022. An attempt was made for identification and evaluation of PSRB & ZnSRB isolates from the wheat rhizosphere from Jammu region (J&K UT). *In vitro* experiment was carried out to isolate and characterize the PSRB & ZnSRB isolates for various PGPR activities. All the standard procedures were followed for the micro biological parameters and soil analysis.

Biochemical characterization was performed for isolates viz. Citrate utilization, Urease, Nitrate reduction, and H₂S production and based on these biochemical tests, these were subjected to identification. The tests results were interpreted and placed in group VI and VIII by referring to the separation outlined in Bergey's Manual of Systematic Bacteriology and confirms the dominance of genera *Staphylococcus*, *Pseudomonas* and *Methylobacterium*.

Qualitative estimation of phosphate solubilization activity of PZn and ZnP isolates in PVK agar plates revealed high phosphate solubilization index of (0.50) & (0.66) respectively at 7th and 9th day with PZn-2 isolate. Quantitative estimation of phosphate solubilization 0.651 µg/ml in PVK broth and ZnS broth was recorded highest with PZn-2 isolate at 13th day interval.

Qualitative estimation of zinc solubilization activity of ZnP and PZn isolates in ZnS agar plates revealed zinc solubilization index (SI) of ZnP-1 isolate and was found to be highest as 0.35 and 0.55 at 7th and 9th day respectively among all the isolates. The maximum Zn solubilization was observed at 9th day. The solubilization efficiency enhanced with successive days of incubation. Quantitative estimation of zinc solubilization of ZnP and PZn isolates in PVK broth and ZnS broth was recorded highest in ZnP-1 isolate with 9.50 µg ml⁻¹ & 42.25 µg ml⁻¹ at 9th and 13th day respectively. The study reveals that bacterial isolates exhibited maximum solubilization activity between 9th to 13th day for phosphate and zinc solubilization respectively.

Pot trial was laid out in factorial CRD with four replications and fifteen treatment combinations. The soil used for experiment was clayey loam in texture and slightly alkaline in nature. The treatments consisted of P₀(control), P₁(TCP), P₂(ZnCO₃) and broth treatment B₀(control), B₁(PZn-1 isolate), B₂(PZn-2 isolate), B₃(ZnP-1 isolate) and B₄(consortium). Application of TCP, ZnCO₃ and consortium treatments showed significant increase on available nutrients in rhizospheric soil of wheat crop. Non-significant decrease in pH was recorded in all stage of wheat growth. Highest available N with (91.43 mg kg⁻¹) at tillering stage, (92.74 mg kg⁻¹) at earhead initiation stage, available P with (17.71 mg kg⁻¹) at tillering stage, (19.35 mg kg⁻¹) at earhead initiation stage, available K with (91.48 mg kg⁻¹) at tillering stage, (93.66 mg kg⁻¹) at earhead initiation stage and available Zn with (1.63 mg kg⁻¹) at tillering stage, (1.70 mg kg⁻¹) at earhead initiation stage were recorded with treatment P₁ & Consortium. P₁ amended PZn and ZnP isolates consortium showed significant increase in SMBC and dehydrogenase activity. Highest SMBC with (92.53 µg⁻¹soil) at tillering stage and (93.97 µg⁻¹ soil) at earhead initiation stage and highest dehydrogenase activity with (57.82 µg-TPF g⁻¹soil hr⁻¹) at tillering stage, (59.10 µg-TPF g⁻¹soil hr⁻¹) at earhead initiation stage were recorded in treatment P₁. Maximum grain yield with (18.56 g pot⁻¹) and N uptake with (20.11 g pot⁻¹), P uptake with (4.73 g pot⁻¹), K uptake with (4.87 g pot⁻¹) and Zn uptake with (27.25 g pot⁻¹) was recorded in treatment P₁, P₂ & B₄ (consortium) in wheat crop as compared to control.

Based on present experimental results from this investigation it was concluded that among the identified, PZn-2 isolate proved to be efficient strain in phosphate solubilization whereas ZnP-1 isolate was most efficient among all isolates for zinc solubilization in wheat crop. Further soil amended with TCP, ZnCO₃ along with consortium and sole use of single isolate resulted in better proliferation of soil microbes and improved rhizosphere soil nutrients and better yield in wheat crop.

Conclusion

- ❖ Based on experimental results from the investigation it was concluded that, PZn-2 isolate proved to be efficient strain in phosphate solubilization whereas ZnP-1 isolate was more efficient among all isolates for zinc solubilization in wheat crop.
- ❖ The results indicated that soil application of consortium amended with TCP and ZnCO₃ and also the soil application of single inoculation of isolates amended with

TCP and ZnCO₃ improved the PGPR activities of wheat crop under pot study. This might be due to mechanism of acidification, chelation and lowering the soil pH.

- ❖ Soil application of single inoculation of isolates as well as consortium amended with TCP and ZnCO₃ improved the rhizospheric properties of wheat crop. The reason is attributed to the fact that synergistic effect of TCP and ZnCO₃ along with isolates resulted in increased nutrient availability which in turn improved rhizospheric properties.
- ❖ Further, the isolation of microorganisms from the rhizosphere and characterizing these strains for plant growth promoting traits could be an alternative strategy for ecofriendly and site-specific nutrient management.
- ❖ Also, in phosphorus and zinc responsive crops PZn and ZnP can be applied as biofertilizers after thirteenth day of incubation in order to obtain maximum crop growth and development. Zn-P isolates can be applied after seventeenth day of incubation for maximum solubilization of zinc by soil microbes. The study can be explored for sustainable soil and plant health in future.

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CERTIFICATE-IV

Certified that all necessary corrections as suggested by the external examiner and advisory committee have been duly incorporated in the thesis entitled "**Isolation, Identification and Evaluation of phosphate and zinc solubilizers in wheat rhizosphere**", submitted by **Mr. Ravi Kumar**, Registration No. **J-20-M-762**.



Major Advisor
Dr. Renu Gupta
Associate Professor
Soil Science and Agricultural Chemistry

Place: Jammu

Date: 25/10/2022



25/10/22

Head of the Division

Vita

VITA

Name of the student : Mr. Ravi Kumar
Father's Name : Late Sh. Rattan Chand
Mother's Name : Smt. Geeta Devi
Nationality : Indian
Date of birth : 01-05-1997
Address : R/O Surni, Tehsil- Ramnagar, District- Udhampur, Jammu and Kashmir(UT), India (182122)
Email ID : tremendousravi60@gmail.com

EDUCATIONAL QUALIFICATIONS

Bachelor's Degree : B. Sc. (Hons) Agriculture
University and year of award : Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu(SKUAST-J), 2020
OGPA : 7.54/10
Master's Degree : M. Sc. Agriculture
Soil Science and Agricultural Chemistry
OGPA : 8.48/10