



# Comparative Effectiveness of KSB, Chemical K Fertilizer and Biotite on Soil K-fractions and Rhizospheric Microbial Activities under Maize Crop (*Zea mays* L)

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

Soils contain more K than any other nutrient; nevertheless, most of the K is inaccessible for plant absorption. In crop production, chemical fertilizers have been associated with several unfavorable outcomes that have a significant detrimental influence on environmental sustainability. Potassium-solubilizing bacteria (KSB) solubilize K-bearing minerals and transform insoluble K into soluble forms, which can be absorbed by plants. Therefore, solubilizing insoluble K in a plant-available pool using K-solubilizing bacteria could be an alternative and viable way to sustain crop production and maintain good soil health. A field experiment was conducted to quantify the changes in rhizospheric K fractions and soil microbial activity in maize crops. The treatments comprised of T<sub>1</sub>: Control; T<sub>2</sub>: KSB<sub>1</sub>; T<sub>3</sub>: 50% RDK; T<sub>4</sub>: 75% RDK; T<sub>5</sub>: T<sub>5</sub> 75 % RDK+25% through Biotite+ KSB; T<sub>6</sub>: 75% RDK + 25% Biotite; T<sub>7</sub>: 50% RDK + 50% Biotite + KSB<sub>1</sub>; T<sub>8</sub>: 50% RDK + 50% Biotite; T<sub>9</sub> 100% RDK. In this study, it was found that combining 75% RDK+25% RDK with biotite and KSB resulted in a better crop yield than using 75% RDK+25% RDK through biotite alone. The recommended dose of potassium (RDK), microbes introducing biotite into soil, affects K dynamics in soil by improving water-soluble, exchangeable, non-exchangeable, and total K pools compared to fertilizer K. Therefore, bio-intervention with waste mica could be an effective and viable method for solubilizing insoluble K into a soluble form, which can be used as a K fertilizer to maintain crop yields and soil K.

*Keywords: Seed inoculation of KSB; biotite; fertilizer K soil microbial population; enzymatic activities; potassium fraction.*

## 1. INTRODUCTION

Potassium is a crucial nutrient for plant growth in soil; however, its availability to plants poses a significant challenge. Although a substantial quantity of potassium (K) is present in most soils, not all reserves are accessible for plant uptake. In the 1980s, Martin and Sparks (1985) identified four distinct forms of K in soil: water-soluble, exchangeable, non-exchangeable, and mineral K. Assessing the availability of K in soil necessitates an understanding of how the soil processes these different forms of K within the Earth's crust, which constitutes 1.9% of its surface (Tisdale et al., 1985). Potassium is indispensable for plant growth and development and performs several critical functions, including enzyme activation, water balance regulation, energy production, nutrient transport, and starch and protein synthesis. Sufficient supply of K is vital for the sustainability and productivity of agriculture. Given India's lack of commercial-grade potash reserves, the country relies heavily on imported potassic fertilizers. During a specific growing season, plants require substantial amounts of K from the soil solution phase to support their growth, necessitating constant renewal of K in the soil to maintain plant health (Singh and Agarwal, 2004). Exchangeable and non-exchangeable K contributed significantly more to crop development than did exchangeable K during the early stages. Nutrient fractionation is commonly employed to determine

potash levels in soil fractions or to measure various elements present in soils (Shuman, 1985). The primary focus for plants is the soil solution or water-soluble K. Mechanisms, such as leaching with percolation water, movement, diffusion to plant roots, ionization, and dissociation, facilitate the availability of K to plants. A higher concentration of potassium-solubilizing bacteria (KSB) is typically found in the rhizosphere than in non-rhizosphere soil, enhancing the transition of K in soil (Padma and Sukumar, 2015). Certain rhizospheric bacteria release potassium from soil K-minerals and play a pivotal role in the natural K cycle. By incorporating KSB into the soil and crop nutrients, KSB can solubilize insoluble K, making it available to plants. Additionally, KSB can serve as an efficient source of K biofertilizer, maintain soil resources, and sustain crop production.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site and Details

The study site in the at (25°18' N and 80°36' E, at 128.93 m above MSL) the experiment plot was conducted in the department of Soil Science and Agricultural Chemistry, Banaras Hindu University (BHU), Varanasi, India. The field experiment was conducted for two years (2019–2020 kharif season, July–November each year) under maize cultivation. The recommended doses of NPK were the primary nutrients required for maize

cultivation, given as RDF (100:40:40 kg N: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O ha<sup>-1</sup>) in each treatment, except for the control (uninoculated). Various combinations of K fertilizers were applied through the MOP and Biotite. KSB<sub>1</sub>- *A. tumefaciens* strain OPVS 07 (KJ410665) was applied as a seed treatment, with nine treatments (T<sub>1</sub> to T<sub>9</sub>) replicated three times and laid out in a completely randomized block design. The nine treatments comprised of T<sub>1</sub>: Control; T<sub>2</sub>: KSB<sub>1</sub>; T<sub>3</sub>: 50% RDK; T<sub>4</sub>: 75% RDK; T<sub>5</sub>: 75% RDK + 25% Biotite + KSB<sub>1</sub>; T<sub>6</sub>: 75% RDK + 25% Biotite; T<sub>7</sub>: 50% RDK + 50% Biotite + KSB<sub>1</sub>; T<sub>8</sub>: 50% RDK + 50% Biotite; T<sub>9</sub>: 100% RDK. (Table 1) shows the important soil parameters at the start of the trial. It should be noted that the experimental site had a consistent topography and texture. In addition to removing excess water from the experiment, proper drainage facilities were employed to remove any excess water.

**Table 1. Initial soil characteristics of experimental field**

Soil properties	Value
pH	6.9
EC (dS m <sup>-1</sup> )	0.35
Bulk density (Mg M <sup>-3</sup> )	1.43
Organic carbon (g kg <sup>-1</sup> )	0.39
Nitrogen (kg ha <sup>-1</sup> )	181.12
Phosphorus (kg ha <sup>-1</sup> )	13.92
Potassium (kg ha <sup>-1</sup> )	151.48

## 2.2 Collection/Processing of Soil Samples

Rhizosphere soil sample (0-15 cm) were collected from maize crops at 30, 60, and 90 days. Plants were uprooted, and after thorough gentle shaking (3-4 times), the adhered rhizospheric soil samples were collected in a container. The collected moist rhizosphere samples were transported to the laboratory and divided into two parts. One half was air-dried, passed through a 2 mm filter, and kept in high-quality polythene containers for subsequent chemical analysis. The second half was stored in a refrigerator at 4°C and used for microbiological analysis, such as the estimation of microbial density and enzymatic activities.

## 2.3 Soil Potassium Fraction Analysis

Soil samples were analyzed for different forms of K, which was determined by shaking 5 g soil for 5 min with 25 ml 1 N NH<sub>4</sub>OAc at pH 7.0 (Hanway and Heidel, 1952), including Saturation Paste Extract (Pratt, 1982), Exchangeable-K by

centrifugation and decantation (Pratt, 1982), non-exchangeable or fixed-K by boiling nitric acid extraction (Pratt, 1982) and total soil K. [viz., water-soluble K (Jackson, 1973), neutral normal ammonium acetate-extractable K (Pratt, 1965) and 1M boiling HNO<sub>3</sub> acid-extractable K (Pratt, 1965). In all the cases, the K concentration was determined using a flame photometer.

## 2.4 Soil Enzymatic Activities and Microbiological Communities

Enzymatic activity in the rhizospheric soil of maize during different growth periods was also studied. Tetraphenyl tetrazolium chloride (TTC) method was used to determine the activity of dehydrogenase enzymes (Casida et al., 1964). The hydrolytic activity of FDA was analyzed using a spectrophotometric method (Greena et al., 2006). The activity of phosphate in soils was measured using a colorimetric method developed by Tabatabai and Bremner (1969), and the activity of urease in soils was measured using a titrimetric method developed by Tabatabai and Bremner (1971) and a spectrophotometric method (Greena et al., 2006). The populations of soil bacteria, fungi, actinomycetes, and K-solubilizing bacteria were enumerated by the serial dilution plate culture technique using nutrient agar, Martin's Rose Bengal and Ken-Knight, Munaier's medium, and Modified Aleksandrov Medium, respectively.

## 2.5 Assessment of Potassium in Maize Plant

The K content of the maize plant samples was determined using the Flame Photometer method (Jackson, 1973). The digested extract was used directly for flame-photometer determination of potassium. The K content was calculated using a standard curve and expressed as total K (%).

## 2.6 Statistical Analysis and Interpretation of Data

Data obtained throughout the experiment were statistically analyzed using a Randomized Block Design (RBD) and appropriate ANOVA tables. We tested the significance of the treatments using the F-test, and we compared valid differences between the treatments with the critical difference (CD) at a 5% degree of significance, using Gomez and Gomez's methodology.

### 3. RESULTS

#### 3.1 Effect of PSB and Doses of Potassium on Grain Yield, Stubble Yield and K-uptake of Maize Crop

The findings showed that at various growth stages of maize, the highest grain yield, stubble yield, and K-uptake were observed with T<sub>5</sub> i.e. 75% RDK + 25% RDK through Biotite + KSB<sub>1</sub> compared to the control treatment. However, T<sub>7</sub> i.e. 50% RDK + 50% RDK through Biotite + KSB<sub>1</sub> demonstrated a 10% lower yield compared to T<sub>5</sub> (Table 2). However, T<sub>5</sub> followed by T<sub>7</sub> exhibited superior maize grain yield, stubble yield, and K-uptake. These results suggest that using 75% RDK + 25% RDK through Biotite + KSB<sub>1</sub> - inoculated seed treatment can replace 10% of the combination of 50% RDK + 50% RDK through Biotite + KSB<sub>1</sub> without compromising overall yield. In the 2019-2020 pooled data were found approximately 57%, 29%, and 67% higher, specifically, in grain yield, stubble yield, and K-uptake under T<sub>5</sub> compared to T<sub>7</sub>. The graphs of linear regression analysis between grain yield and K uptake by grain (Figs. 1a and 1b) were found to be the best fit in the regression prediction ( $R^2=0.870$  and  $R^2=0.937$ ) in the linear

regression than between stubble yield and K uptake by stubble (Fig. 1c and 1d) ( $R^2=0.750$  and  $R^2=0.754$ ) in 2019 and 2020, respectively.

#### 3.2 Effect of Potassium Solubilizing Bacteria and doses of Potassium on Potassium Fraction in soil at Different Growth Periods of Maize

Soil potassium (K) pools, such as water-soluble K, exchangeable K, non-exchangeable K, and total-K in the soil at 30 and 60 DAS and at harvest of maize differed significantly due to KSB<sub>1</sub> and RDK (Tables 3–4), and all treatments resulted in higher levels of all forms of K compared to the control. All forms of potassium were recorded at T<sub>5</sub> followed by T<sub>7</sub> at 30 DAS, 60 DAS, and at harvest of the maize crop. T<sub>5</sub> caused higher water-soluble, exchangeable, non-Exchangeable, and total potassium, which were ~ 26, 55, 44% and ~ 20,26,27% and ~ 23,22,18% and ~ 14, 16,13% respectively, greater than the control. Treatment T<sub>5</sub> yielded higher K pools in all fractions than T<sub>4</sub> and T<sub>3</sub> at different crop intervals. Treatment T<sub>5</sub> compared with other treatments, such as T<sub>8</sub> and T<sub>9</sub> caused higher exchangeable potassium.

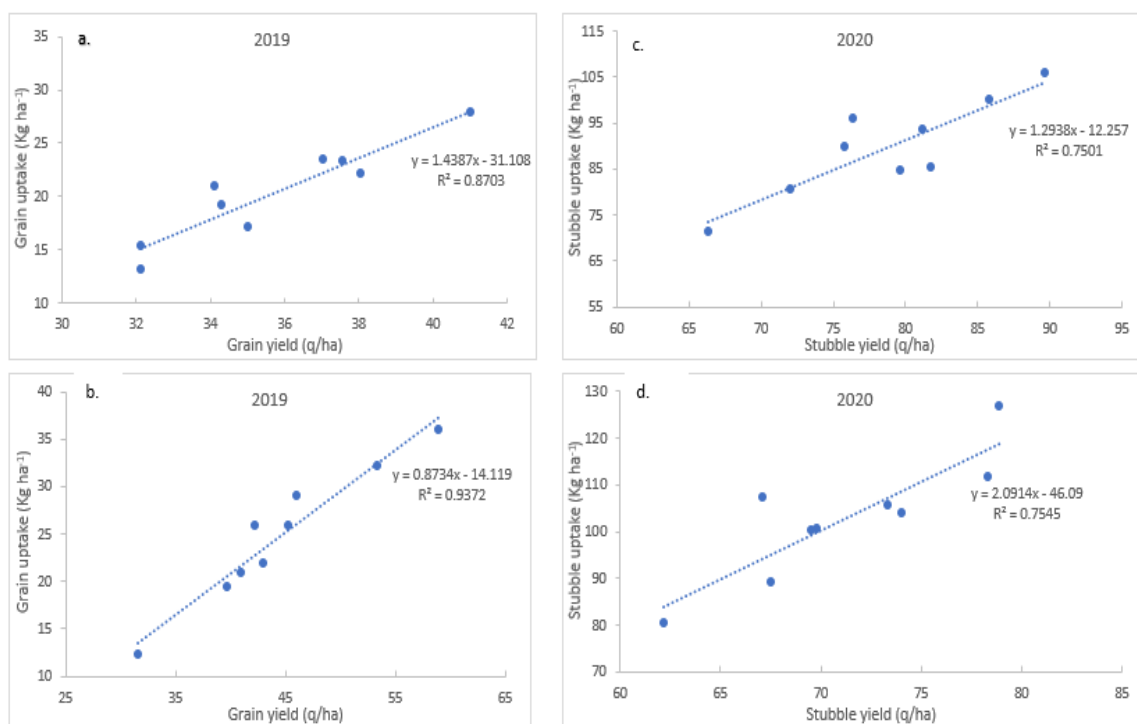


Fig. 1. Relationship between potassium grain uptake and grain yield (a,b) and potassium stubble uptake and stubble yield (c,d) across all treatments

**Table 2. Effect of potassium solubilizing bacteria and doses of potassium on grain yield, stubble yield and potassium uptake (Kg ha<sup>-1</sup>) by maize crop**

Treatments	Grain						Stubble						Total uptake (Kg ha <sup>-1</sup> )		
	Yield q/ha			Uptake (Kg ha <sup>-1</sup> )			Yield q/ha			Uptake (Kg ha <sup>-1</sup> )			2019	2020	Pooled
	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled			
T <sub>1</sub> Control	32.12	31.62	31.87	13.16	12.33	12.75	66.34	62.17	64.25	71.21	80.23	75.72	84.37	92.57	88.47
T <sub>2</sub> KSB	32.12	39.75	35.93	15.41	19.40	17.41	72.02	67.52	69.77	80.37	89.25	84.81	95.78	108.65	102.21
T <sub>3</sub> 50% RDK	35.01	40.96	37.98	17.16	20.95	19.06	79.66	69.83	74.75	84.58	100.67	92.63	101.74	121.62	111.68
T <sub>4</sub> 75% RDK	34.32	42.96	38.64	19.15	21.88	20.52	81.22	74.01	77.62	93.43	103.91	98.67	112.58	125.80	119.19
T <sub>5</sub> 75 % RDK+25% through Biotite+ KSB	41.00	58.99	50.00	27.85	35.99	31.92	89.69	78.91	84.30	105.64	126.71	116.17	133.49	162.69	148.09
T <sub>6</sub> 75% RDK + 25% through Biotite	34.12	42.24	38.18	20.88	25.89	23.38	75.81	69.55	72.68	89.92	100.14	95.03	110.80	126.03	118.42
T <sub>7</sub> 50% RDK+50% through Biotite + KSB	37.57	53.41	45.49	23.32	32.21	27.76	85.79	78.31	82.05	100.11	111.70	105.91	123.42	143.92	133.67
T <sub>8</sub> 50% RDK + 50% through Biotite	38.04	45.30	41.67	22.06	25.82	23.94	81.75	67.14	74.44	85.32	107.28	96.30	107.38	133.11	120.24
T <sub>9</sub> 100% RDK	37.06	46.08	41.57	23.38	28.96	26.17	76.38	73.35	74.86	95.95	105.42	100.69	119.33	134.38	126.86
SEm±	1.72	3.00	2.44	1.26	1.51	1.39	4.36	3.32	3.88	4.77	6.31	5.61	5.05	6.33	5.72
LSD (P=0.05)	8.36	11.67	10.57	10.85	10.57	10.74	9.60	8.07	8.95	9.23	10.64	10.08	7.96	8.59	8.35

**Table 3. Effect of potassium solubilizing bacteria and doses of potassium on water soluble and exchangeable potassium (mg kg<sup>-1</sup>) of soil at different growth periods of maize crop**

Treatments	Water soluble K (mg kg <sup>-1</sup> )									Exchangeable potassium (mg kg <sup>-1</sup> )								
	30 DAS			60 DAS			At harvest			30 DAS			60 DAS			At harvest		
	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled
T <sub>1</sub> Control	13.28	12.66	12.97	15.38	16.13	15.76	13.80	15.36	14.58	103.26	103.72	103.49	103.31	109.33	106.32	104.28	104.44	104.36
T <sub>2</sub> KSB	13.29	13.51	13.40	16.33	17.36	16.84	14.73	16.10	15.42	105.63	105.83	105.73	120.46	117.24	118.85	122.23	118.90	120.56
T <sub>3</sub> 50% RDK	13.48	13.66	13.57	17.31	17.76	17.53	15.69	16.20	15.94	106.02	107.79	106.91	121.92	123.17	122.54	123.73	122.02	122.87
T <sub>4</sub> 75% RDK	14.00	13.84	13.92	17.30	17.80	17.55	15.67	16.80	16.24	106.81	112.57	109.69	122.41	125.00	123.70	124.15	125.47	124.81
T <sub>5</sub> 75 % RDK+25% through Biotite+ KSB	16.14	16.46	16.30	24.23	24.62	24.42	22.44	19.64	21.04	122.94	125.10	124.02	132.54	135.82	134.18	131.74	133.11	132.43
T <sub>6</sub> 75% RDK + 25% through Biotite	15.13	15.60	15.37	20.23	20.65	20.44	18.59	18.21	18.40	113.39	117.43	115.41	126.28	129.00	127.64	128.42	127.73	128.08
T <sub>7</sub> 50% RDK+50%	15.89	16.03	15.96	21.22	22.24	21.73	19.54	19.30	19.42	119.18	121.63	120.40	129.10	133.61	131.35	130.86	132.28	131.57

Treatments	Water soluble K (mg kg <sup>-1</sup> )									Exchangeable potassium (mg kg <sup>-1</sup> )									
	30 DAS			60 DAS			At harvest			30 DAS			60 DAS			At harvest			
	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	
through Biotite + KSB																			
T <sub>8</sub> 50% RDK + 50% through Biotite	14.11	14.00	14.06	17.26	17.96	17.61	15.62	17.53	16.57	108.06	113.85	110.95	124.55	126.27	125.41	126.97	127.29	127.13	
T <sub>9</sub> 100% RDK	15.31	15.94	15.63	20.22	21.29	20.76	18.56	18.56	18.56	116.69	118.11	117.40	128.60	130.77	129.68	130.78	130.17	130.47	
SEm±	0.54	0.60	0.19	0.82	0.88	0.14	0.79	0.83	0.72	4.27	4.48	1.04	4.82	5.02	1.27	5.19	5.45	0.80	
LSD (P=0.05)	1.61	1.80	0.61	2.44	2.64	0.45	2.36	2.49	2.34	12.80	13.42	3.41	14.46	15.06	4.15	15.57	16.33	2.60	

**Table 4. Effect of potassium solubilizing bacteria and doses of potassium on non-exchangeable potassium and total potassium of soil at different growth periods of maize crop**

Treatments	Non- exchangeable potassium (mg kg <sup>-1</sup> )									Total potassium (mg kg <sup>-1</sup> )								
	30 DAS			60 DAS			At harvest			30 DAS			60 DAS			At harvest		
	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled
T <sub>1</sub> Control	318.5	335.0	326.8	329.8	339.2	334.5	330.9	340.4	335.7	5443	5646	5545	5582	5699	5641	5596	5714	5655
T <sub>2</sub> KSB	326.5	344.0	335.3	340.4	345.5	342.9	339.5	359.3	349.4	5541	5758	5650	5713	5777	5745	5703	5948	5825
T <sub>3</sub> 50% RDK	327.9	345.7	336.8	342.4	358.2	350.3	340.3	361.0	350.6	5558	5779	5669	5739	5934	5836	5713	5968	5841
T <sub>4</sub> 75% RDK	335.5	354.0	344.8	351.7	364.9	358.3	349.8	362.5	356.2	5653	5882	5768	5853	6017	5935	5831	5987	5909
T <sub>5</sub> 75 % RDK+25% through Biotite+ KSB	380.2	400.0	390.1	401.0	413.0	407.0	388.0	405.0	396.5	6207	6452	6330	6464	6613	6538	6303	6514	6408
T <sub>6</sub> 75% RDK + 25% through Biotite	354.3	373.5	363.9	370.6	380.5	375.6	370.2	374.5	372.3	5886	6123	6004	6088	6210	6149	6082	6136	6109
T <sub>7</sub> 50% RDK+50% through Biotite + KSB	377.9	397.4	387.6	394.6	392.1	393.3	387.8	395.6	391.7	6178	6420	6299	6385	6354	6370	6301	6397	6349
T <sub>8</sub> 50% RDK + 50% through Biotite	347.6	366.3	357.0	364.3	376.7	370.5	361.2	372.5	366.9	5803	6035	5919	5957	6163	6060	5971	6112	6042
T <sub>9</sub> 100% RDK	359.0	378.4	368.7	375.7	385.4	380.5	373.1	384.0	378.6	5944	6185	6064	6150	6271	6211	6119	6254	6187
SEm±	13.8	13.9	0.6	14.2	14.5	2.7	12.9	12.3	2.7	171	172	7	177	179	33	160	152	34
LSD (P=0.05)	41.3	41.7	1.8	42.7	43.4	8.8	38.8	36.8	9.0	512	517	22	529	538	109	480	456	111

**Table 5. Effect of potassium solubilizing bacteria and doses of potassium on bacterial and fungal population at different growth periods of maize crop**

Treatments	Bacterial population (cfu × 10 <sup>6</sup> g <sup>-1</sup> )									Fungal population (cfu × 10 <sup>4</sup> g <sup>-1</sup> )								
	30 DAS			60 DAS			At harvest			30 DAS			60 DAS			At harvest		
	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled
T <sub>1</sub> Control	16.75	15.23	16.33	21.03	21.12	21.08	19.23	20.25	19.74	15.81	15.67	15.74	20.88	21.12	21.00	19.96	20.58	20.27
T <sub>2</sub> KSB	16.69	15.15	16.81	21.47	21.36	21.41	20.34	20.54	20.44	16.62	16.33	16.48	21.86	21.33	21.60	20.17	21.67	20.92
T <sub>3</sub> 50% RDK	17.68	18.27	17.42	21.53	21.83	21.68	20.43	20.62	20.53	16.98	16.33	16.66	21.89	21.33	21.61	20.29	22.04	21.17
T <sub>4</sub> 75% RDK	16.06	16.53	18.01	21.95	21.84	21.90	21.56	21.73	21.64	17.45	17.34	17.39	21.93	21.67	21.80	21.16	22.33	21.74
T <sub>5</sub> 75% RDK+25% through Biotite+ KSB	19.30	18.88	20.74	25.05	25.46	25.26	23.50	23.47	23.49	19.16	19.33	19.24	24.00	24.58	24.29	23.75	23.65	23.70
T <sub>6</sub> 75% RDK + 25% through Biotite	19.90	17.98	19.34	22.38	23.24	22.81	21.85	22.19	22.02	17.50	17.67	17.59	22.80	22.68	22.74	22.12	22.64	22.38
T <sub>7</sub> 50% RDK+50% through Biotite + KSB	19.20	17.32	19.53	24.39	24.59	24.49	22.81	23.36	23.08	18.78	18.17	18.47	23.58	23.26	23.42	23.14	23.69	23.41
T <sub>8</sub> 50% RDK + 50% through Biotite	19.70	20.06	19.95	22.24	22.65	22.44	21.65	22.06	21.85	17.47	17.33	17.40	22.18	22.57	22.38	21.33	22.69	22.01
T <sub>9</sub> 100% RDK	19.45	22.15	20.16	22.80	24.01	23.40	22.01	22.63	22.32	18.25	17.92	18.08	23.47	22.69	23.08	22.14	22.69	22.42
SEm±	0.86	0.98	0.72	0.83	0.88	0.22	0.81	0.73	0.15	0.65	0.68	0.15	0.63	0.64	0.23	0.82	0.59	0.30
LSD (P=0.05)	2.57	2.93	2.35	2.50	2.62	0.71	2.44	2.19	0.51	1.94	2.04	0.48	1.89	1.93	0.76	2.45	1.78	0.97

**Table 6. Effect of potassium solubilizing bacteria and doses of potassium on actinomycetes and K-solubilizing bacterial population at different growth periods of maize crop**

Treatments	Actinomycetes population (cfu × 10 <sup>4</sup> g <sup>-1</sup> )									K-solubilizing bacterial population (cfu × 10 <sup>4</sup> g <sup>-1</sup> )								
	30 DAS			60 DAS			At harvest			30 DAS			60 DAS			At harvest		
	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled
T <sub>1</sub> Control	14.69	14.67	14.68	15.81	15.32	15.57	15.98	15.67	15.83	12.37	12.33	12.35	13.99	13.67	13.83	11.83	12.18	12.01
T <sub>2</sub> KSB	15.57	14.88	15.22	16.76	17.33	17.04	16.50	16.10	16.30	12.69	12.37	12.53	14.61	14.67	14.64	12.75	12.98	12.87
T <sub>3</sub> 50% RDK	16.04	15.52	15.78	17.94	18.52	18.23	16.56	16.09	16.33	12.84	12.67	12.75	14.83	14.83	14.83	12.84	13.33	13.09
T <sub>4</sub> 75% RDK	15.64	15.32	15.48	18.83	18.68	18.76	16.74	16.33	16.54	13.12	13.13	13.13	14.98	15.33	15.16	13.51	13.67	13.59
T <sub>5</sub> 75% RDK+25%	17.13	16.57	16.85	20.55	20.40	20.47	18.45	18.67	18.56	14.21	14.76	14.48	16.94	16.52	16.73	14.47	15.90	15.19

Treatments	Actinomycetes population (cfu × 10 <sup>4</sup> g <sup>-1</sup> )									K-solubilizing bacterial population (cfu × 10 <sup>4</sup> g <sup>-1</sup> )									
	30 DAS			60 DAS			At harvest			30 DAS			60 DAS			At harvest			
	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	2019	2020	Pooled	
through Biotite+ KSB																			
T <sub>6</sub> 75% RDK + 25% through Biotite	15.91	15.31	15.61	18.93	19.33	19.13	16.96	16.67	16.82	13.42	13.67	13.55	16.18	16.17	16.17	14.16	15.24	14.70	
T <sub>7</sub> 50% RDK+50% through Biotite + KSB	16.74	16.47	16.61	19.56	19.68	19.62	17.41	17.67	17.54	13.91	14.67	14.29	16.50	16.47	16.49	14.37	15.67	15.02	
T <sub>8</sub> 50% RDK + 50% through Biotite	15.79	15.32	15.55	18.90	18.66	18.78	16.94	16.67	16.80	13.37	13.44	13.41	15.81	15.33	15.57	13.70	14.19	13.94	
T <sub>9</sub> 100% RDK	16.09	16.33	16.21	19.41	19.51	19.46	17.02	16.69	16.85	13.58	14.33	13.96	15.88	15.46	15.67	14.31	15.37	14.84	
SEM±	0.41	0.43	0.15	0.78	0.84	0.19	0.42	0.56	0.14	0.36	0.58	0.20	0.56	0.57	0.14	0.55	0.60	0.24	
LSD (P=0.05)	1.24	1.28	0.49	2.35	2.53	0.61	1.27	1.67	0.44	1.09	1.75	0.64	1.69	1.70	0.46	1.65	1.79	0.79	

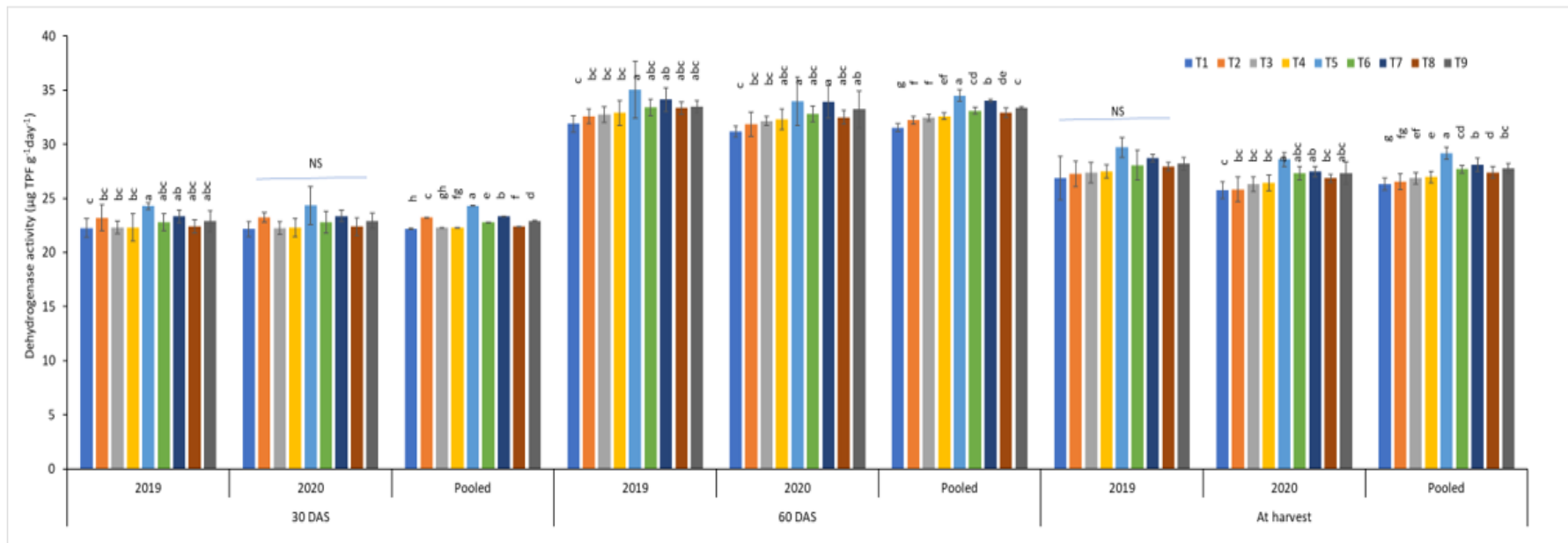


Fig. 2. Effect of potassium solubilizing bacteria and doses of potassium on dehydrogenase activity in rhizospheric soil at different growth periods of maize crop

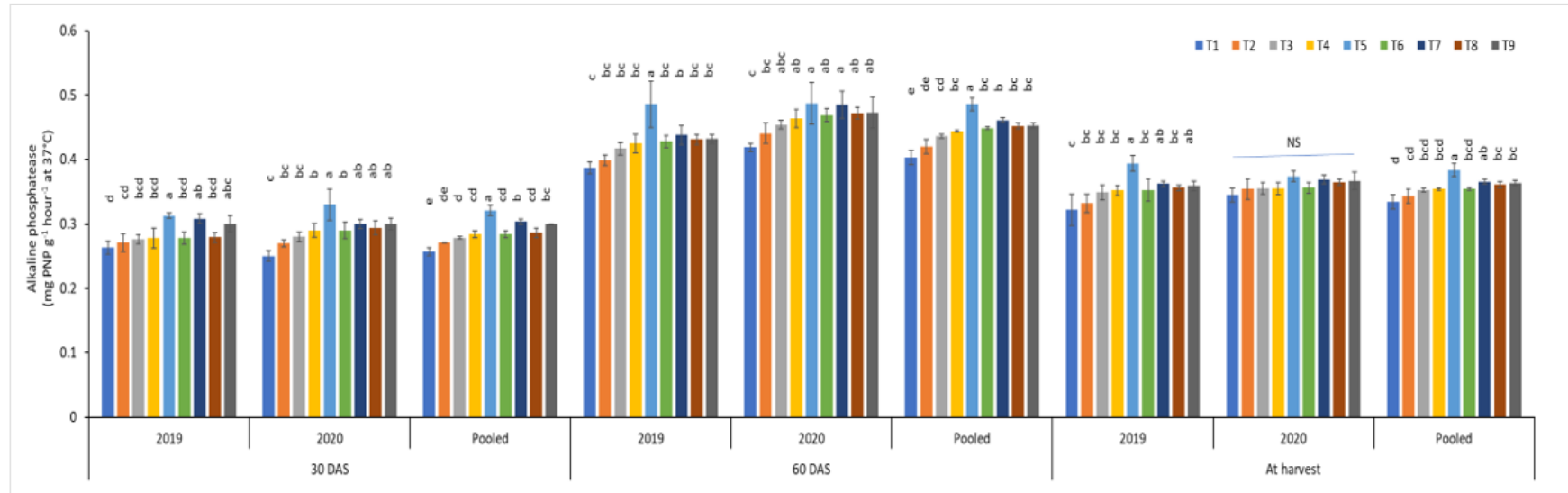


Fig. 3. Effect of potassium solubilizing bacteria and doses of potassium on alkaline phosphatase activity in rhizospheric soil at different growth periods of maize crop

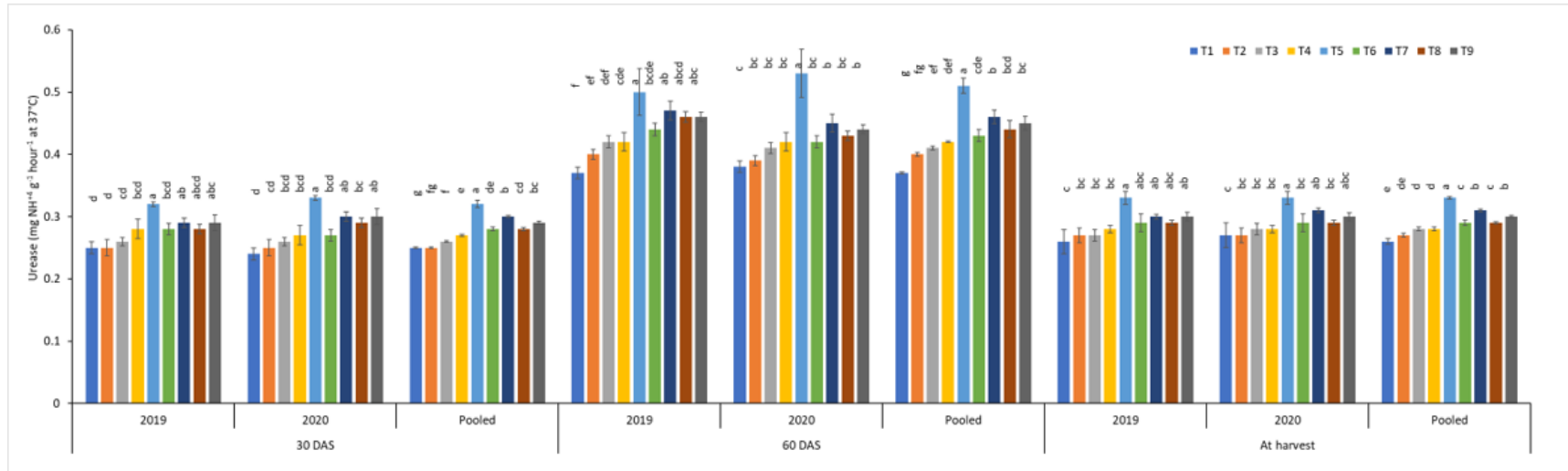
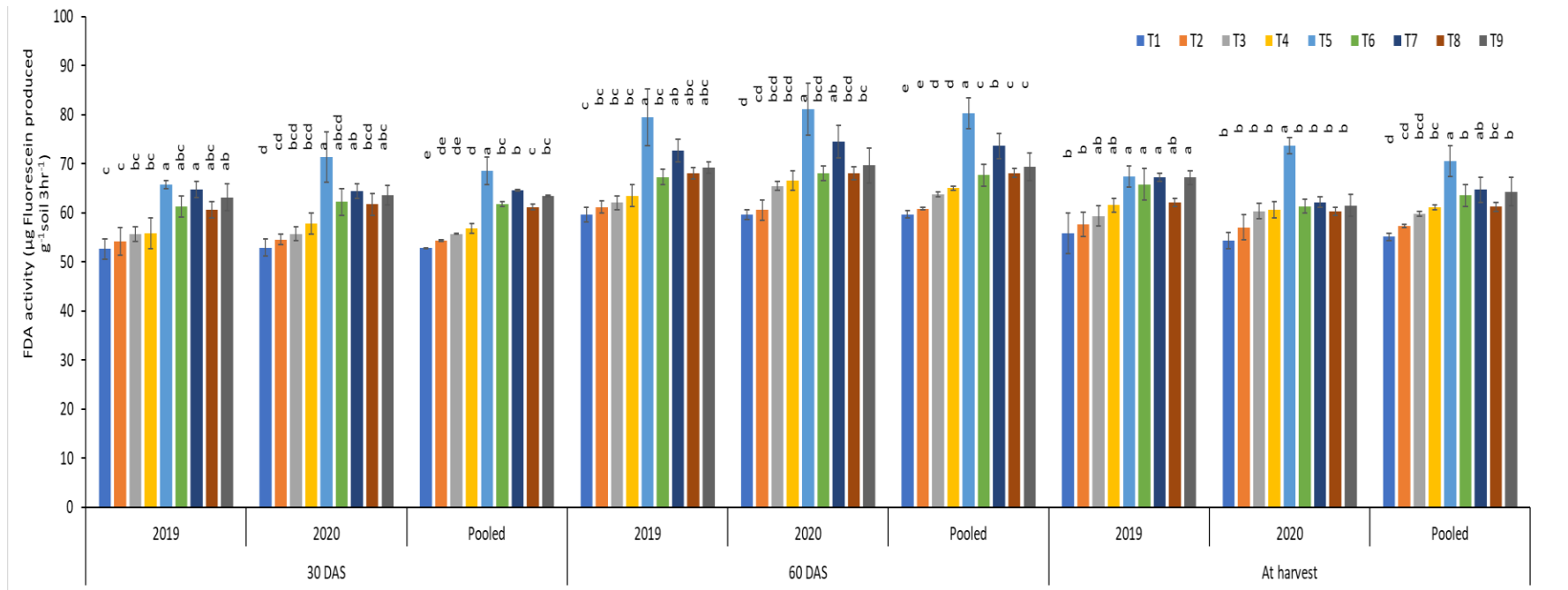
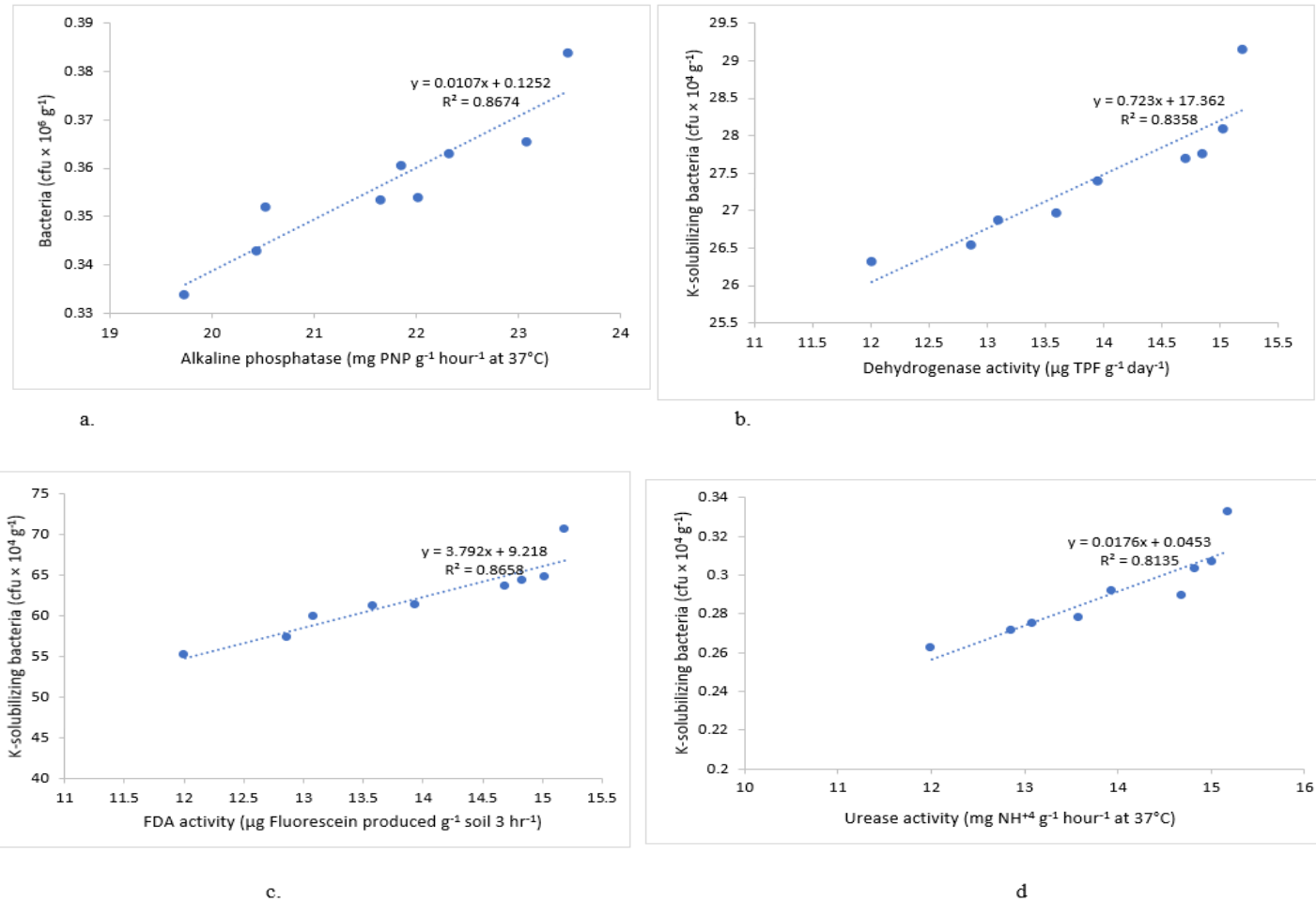


Fig. 4. Effect of potassium solubilizing bacteria and doses of potassium on Urease activity in rhizospheric soil at different growth periods of maize crop



**Fig. 5. Effect of potassium solubilizing bacteria and doses of potassium on FDA activity in rhizospheric soil at different growth periods of maize crop**



**Fig. 6.** Relationship between soil microbes and soil enzymatic activities (a. Bacteria and alkaline phosphatase; b. K-solubilizing bacteria and dehydrogenase activity; c. K-solubilizing bacteria and FDA activity and d. K-solubilizing bacteria and urease activity) across all treatments

### 3.3 Soil Enzymatic Activities and Microbiological Communities

The application of KSB and K had a substantial effect on the soil enzyme activity and microbial populations. All treatments showed higher dehydrogenase activity than that of the control (Fig. 2). Treatment with T<sub>5</sub> followed by T<sub>7</sub> resulted in the highest dehydrogenase activity. These treatments had ~10, 9, 11% and ~6, 8,7% respectively increase in dehydrogenase activity at 30 DAS, 60 DAS, and at harvest of maize crop compared to the control. Although the soil dehydrogenase activity at 30 DAS in both 2019 and 2020 was not significant, it was not significant in 2019 at harvest. Phosphatase activity was ~ 25, 21 and 15% higher for T<sub>5</sub> and 18,14 and 10% higher for T<sub>7</sub> and at harvest of maize crop, respectively, over control (Fig. 3). The phosphatase activity at harvest in 2020 was not significant. The activity of urease followed the same trend as that of the other enzymes (Fig. 4). At different crop intervals, treatment T<sub>5</sub> exhibited higher urease activity than T<sub>4</sub> and T<sub>3</sub>. Treatment T<sub>5</sub> followed by T<sub>7</sub> caused highest FDA activity, which was ~30, 35, 28%, and 22, 23, 17%, respectively, greater than the control (Fig. 5). The microbial population was significantly enhanced by the application of KSB and potassium doses. The maximum colony forming units (cfu) of bacterial, fungal, actinomycete, and K-solubilizing bacterial populations were recorded with T<sub>5</sub> followed by T<sub>7</sub> at 30 DAS, 60 DAS, and at harvest, respectively of maize crop (Tables 5-6). The graphs of linear regression analysis between bacterial populations and alkaline phosphatase (Fig. 6a) showed the best fit in the regression prediction with R<sup>2</sup>=0.867, followed by the K-solubilizing bacterial population and different soil enzymes (FDA, dehydrogenase, and urease activity) (Fig. 6b, 6c and 6d) (R<sup>2</sup>=0.866, R<sup>2</sup>=0.836, and R<sup>2</sup>=0.814). It was found out that almost all the parameters were positively and significantly correlated with each other at P<0.05%.

## 4. DISCUSSION

### 4.1 Effect of PSB and doses of Potassium on Potassium Fraction in Soil

When mica was inoculated with KSBs, the microbes solubilized non-exchangeable and structural K through the formation of organic acids such as oxalic and citric acids, and the amount became more accessible because of the greater rhizosphere impact (Rovira, 1965; Meena

et al., 2014). It is clear that the application of 75% RDK +25 % RDK through Biotite yielded higher exchangeable potassium than the application of 50% RDK+50% RDK through Biotite at 30 DAS, 60 DAS, and at harvest of crop. As observed by Jatav and Dewangan (2012), there was a significant positive correlation between the soil K fractions. Soil K was classified in the following order: non-exchangeable K, exchangeable K, accessible K, and water-soluble K. Soil pH was positively and significantly correlated with water-soluble, exchangeable, and available K. Treatment T<sub>5</sub> i.e. 75 % RDK+25% through Biotite+ KSB) gave higher non-exchangeable potassium than T<sub>4</sub> i.e. 75% RDK) and T<sub>3</sub> i.e. 50% RDK) at different intervals of crop. Treatment T<sub>5</sub> compared with other treatments such as T<sub>8</sub> and T<sub>9</sub> caused higher non-exchangeable potassium. Treatment T<sub>6</sub> i.e., 75% RDK +25 % RDK through Biotite) gave higher non-exchangeable potassium than T<sub>8</sub> (50% RDK+50 % RDK through Biotite). The release of non-exchangeable K from feldspars has also been attributed to the exudation of low-molecular-weight organic acids, particularly citric and oxalic acids, from the roots (Wang *et al.* 2000; Moritsuka *et al.*, 2004, Meena *et al.*, 2014, 2015). Potassium solubilizers have been proven to be more efficacious under stress conditions. Pal (2014) reported that the difference in the concentration gradient between mineral K and other pools present in the soil may have compelled the release of some of the interlayer lattice K. However, this release is very difficult because K occurs in the interstices of the Si, Al-O framework of the crystal lattice and is bound tightly by covalent bonds. Das (2015) showed that the action of KSBs is extensive, because K is first released by water and subsequently by weak acids at a faster pace. However, when weathering progresses, an envelope of Si-Al-O residue forms around the unweathered core. This layer slows the rate of K loss from the mineral, thus protecting it from further degradation.

### 4.2 Effect of Potassium Solubilizing Bacteria and doses of Potassium on Soil Enzymatic Activities and Microbial Populations

Soil dehydrogenase activity is an important indicator of microbial activity in the soil as it reflects the total range of oxidative activity of the soil microflora. The findings of this study suggest that the enhanced growth of crops, as a result of the use of KSB and K doses, increased microbial activities and dehydrogenase activity in the

rhizosphere. This, in turn, may have contributed to the availability of food for crop growth. These results suggest that the use of potassium-solubilizing bacteria and appropriate doses of potassium can improve microbial activity and enhance the growth of crops in maize rhizosphere soils. In this study, there was a positive interaction between the K-solubilizing bacterial population in the soil and dehydrogenase ( $r = 0.83^{**}$ ), FDA ( $r = 0.86^{**}$ ), and urease ( $r = 0.81^{**}$ ) activities in the soil. Therefore, the application of 50% RDK +50 % RDK through Biotite gave higher alkaline phosphatase activity than the application of 75% RDK+25 % RDK through Biotite at 30 DAS, 60 DAS, and at harvest of crop. The maximum release of root exudates may be linked to the higher microbial activity in the rhizosphere. The rhizosphere microorganism population in the soil supplies multiple nutrients and energy to microorganisms through root exudates (Wang et al., 2000). Therefore, the application of 75% RDK +25 % RDK through Biotite gave higher FDA activity than the application of 50% RDK+50% RDK through Biotite at 30 DAS, 60 DAS, and at harvest of crop. Based on their study, Aseri and Tarafdar (2006) found that FDA hydrolyzable enzyme activity is a better biomarker for arid soils than dehydrogenase activity. Similar results have been reported by Patle et al., (2018), who studied the overall potential of microbial activity and various changes in soil biological characteristics to preserve soil health and quality. Sugumaran and Janarthanam (2007) reported that the application of KSB on different minerals, including mica, increased the total number of bacterial populations, and similarly found positive interactions between bacterial populations in soil and alkaline phosphatase ( $r = 0.86^{**}$ ) activity in the soil. This showed that the fungal population increased irrespective of treatment because of colonization, more fruiting bodies under greater deposition, and inoculation with KSB. According to Guan et al. (2011), the use of inorganic and organic fertilizers simultaneously increased fungal density, according to Guan et al., (2011). Additionally, T<sub>5</sub> showed a higher population of actinomycetes than T<sub>8</sub> and T<sub>9</sub>. Treatment T<sub>6</sub>, which involved the application of 75% RDK along with 25% RDK through biotite, resulted in a higher population of actinomycetes compared to T<sub>8</sub>, which involved the application of 50% RDK along with 50% RDK through biotite- and potassium solubilizing bacterial isolates. A previous study conducted by Mukherjee and Gaur (1985) found that incorporating organic matter into the soil of a cropping field resulted in

a higher population of actinomycetes due to increased root activity. All treatments caused significantly more K-solubilizing bacterial populations at different growth periods than the control. The minimum K-solubilizing bacterial population was observed in the control (T<sub>1</sub>) at 30 and 60 d, and harvest of the crop. Kumari et al. (2018) found that soil microbial populations were improved by the use of microbial inoculants or biofertilizers, which increased the nutrient availability. The microbial population of the soil was significantly increased when the NP was 100% + 75% K + Agrobacterium sp. was added. This meant that NPK was equivalent to 100%NPK, so that the soil was enriched and improved and that chemical effects were reduced as a result (Nannipieri et al., 1990).

### 4.3 Effect of PSB and doses of Potassium on Grain Yield, Stubble Yield and K-uptake of Maize Crop

Data revealed that K content and uptake by maize crops due to treatment T<sub>5</sub> i.e. 75% RDK + 25% RDK through Biotite + KSB was greater than T<sub>7</sub> i.e. 75% RDK + 25% RDK) through Biotite and T<sub>9</sub> i.e. 100% RDK). It is also clear that the combination of inorganic and mineral sources of K, along with KSB, yielded significantly better results. This can be attributed to the solubilization of the mineral form of K, that is, Biotite by KSB, which helps in releasing K from minerals and thus enhances the content and uptake by maize plants. Similar results have been reported by Sheng et al. (2003), Archana et al. (2008), Basak et al. (2009), Kunoto et al. (2010), Chang et al. (2014), Prajapati et al. (2016), and Xiao et al. (2017), (Nihorimbere et al., 2011, Aaronson, 1970, Brady, & Weil, 2002).

## 5. CONCLUSION

Application of T<sub>5</sub>:75% RDK+25% RDK through biotite + KSB isolate yielded better results than 75% RDK+25% RDK through biotite. As a result of bacterial intrusion of biotite, water-soluble, exchangeable, non-exchangeable, and total K pools in soils increase, which influences the K dynamics in soils, making them more accessible to plants. Thus, bio-intervention of waste mica could be an effective and viable way of solubilizing insoluble K and could serve as an efficient source of K fertilizer for the maintenance of crop production and soil K as a natural resource management activity. The KSB isolate

*Agrobacterium tumefaciens* (KJ 410665) can be used to produce biofertilizers in large quantities.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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