

Inoculation of Maize with 1-Aminocyclopropane-1-Carboxylic Acid Containing *Pseudomonas fluorescens* for Enhanced Available Nutrients in Soil of Maize (*Zea mays* L.)

**S. R. Jakhar ^{a*}, N. G. Mitra ^a, R. K. Sahu ^a, S. S. Baghel ^a, B. Yaduwanshi ^a
and J. Bangre ^a**

^a Department of Soil Science and Agricultural Chemistry, J.N. Krishi Vishwa Vidyalyaya, Jabalpur - 482 004 (M.P.), India.

Authors' contributions

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

Article Information

DOI: 10.9734/IJECC/2022/v12i130620

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/83468>

Received 15 November 2021

Accepted 17 January 2022

Published 20 January 2022

Original Research Article

ABSTRACT

A field and lab experiment entitled "Kinetics of 1-Aminocyclopropane-1-carboxylic -deaminase (ACCD) activity of *Pseudomonas fluorescens*, its population growth under different fermentation conditions and performance of the selected isolates on maize" was conducted at Laboratory of Microbes Research & Production Centre (MRPC) and the Research Farm, Department of Soil Science and Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalyaya, Jabalpur, Madhya Pradesh during 2018-19. The results revealed that the available nitrogen (N) content in soil at knee height, tasseling and silking stage, the treatment combination Temperature 28°C and pH 7.2 (T₂₈+pH 7.2) isolates significantly increase the N content in soil with response of 21.5, 17.3 and 14.4% over FUI (209, 196 and 166 kg N ha⁻¹), respectively. Similarly, the isolates of T₂₈+pH 7.2 significantly increase the available phosphorus (P) content at knee height, tasseling and silking stage in soil with response of 31.7, 23.2 and 26.4% over FUI (18.0, 16.4 and 14.4 kg P ha⁻¹). The isolates of T₂₈+pH 7.2 significantly increase the available potassium (K) content in soil at knee height, tasseling and silking stage with response of 14.4, 10.1 and 10.4% over FUI (298, 278 and 264 kg K ha⁻¹).

Keywords: ACC-deaminase; maize; plant growth promoting rhizobacteria; yield.

1. INTRODUCTION

Maize (*Zea mays* L.) is an important crop in temperate and semi-arid regions and one of the three major food staple crops for the world's population. India occupies fifth place in acreage and ranks 10th in production. Globally, maize is known as queen of cereals because it has the highest genetic yield potential among the cereals. The average productivity in India is 1959 kg ha⁻¹ grown in 7.18 million hectares area contributing 14.1 MT of production, and the crop is the third most important food crops after rice and wheat [1]. The state of Madhya Pradesh occupies 13% of the total maize area and contributing equally to the total maize production in the country. Nutritionally, maize contains 60 to 68% starch, 1.2 to 5.7% edible oil and 7 to 15% protein [2].

Many agricultural soils around the world are deficient in plant nutrients. As a result, significant fertilizer requirements pose a significant challenge to sustainable food production. Previously, these plant nutrients could only be obtained through the use of synthetic chemical fertilisers. These chemical fertilisers are quite costly and raise crop production costs. Furthermore, chemical fertilisers degrade soil and endanger the health of both humans and farm animals [3]. It is therefore critical to improve soil fertility while avoiding the negative environmental effects of chemical fertilisers. Microbial inoculants are gaining popularity due to their potential role in improving soil fertility and increasing crop yields and nutrient content. Microbial inoculants are formulations that contain beneficial microorganisms that play an important role in all ecosystems. Microbial inoculants improve nutrient availability to the host plant and promote plant growth when applied to seeds, soil, or seedlings [4,5]. A variety of beneficial soil microorganisms are used as inoculants in current agricultural practises. Among them are *Pseudomonas*, *Azospirillum*, *Azotobacter*, and *Phosphobacterium* [6,7]. Microbial inoculants promote plant growth through a variety of mechanisms, including the production of plant hormones, nutrient supply, and the suppression of various crop pests [8].

2. MATERIALS AND METHODS

The present field investigation was carried out in 2 phases as Component- I (during rainy season

of 2018): Kinetics of population growth of *P. fluorescens* and 1-Aminocyclopropane-1-carboxylic acid deaminase (ACCD) activity of the bacteria under different conditions of fermentation; and Component- II (during winter season of 2019): performance of selected isolates of *P. fluorescens* on maize. Studies of component- I and II were performed at the Laboratory of Microbes Research & Production Centre and the Research Farm, Department of Soil Science & Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur India, (latitude of 23° 13 ' N and longitude of 79° 57' E with an altitude of 393 m above mean sea levels) in the period of Feb-June, 2019. The field soil was deep black soil in texture, having pH, 7.48; organic carbon; 0.53%, available N-233 kg ha⁻¹ and 14.9 kg ha⁻¹ of available P and 209 kg ha⁻¹ of available K, respectively and *P. fluorescens* population (1.35×10⁴ cfu g⁻¹ soil) was recorded) respectively. Under fermentation experiment a local but effective strain of *P. fluorescens* was batch cultured for 13 intervals of time (at 0, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144 hr) with 16 treatments including four temperature regimes (i.e., 25, 28, 31, 34°C), four pH (i.e., 6.7, 7.2, 7.7, 8.2) and their interaction under Randomized block designs (RBD) factorial design. Broth samples were drained out from the fermentator at the scheduled intervals for study on the bacterial population growth and enzyme ACCD activity. Under field study, the 16 isolates derived from the treatments of different temperature and pH with additional 2 controls [Total treatments = 18 (16 isolates + 1 FUI + 1 unfertilized uninoculated (UFUI))] were tried on maize following RBD design with 3 replications. Data were statistically treated by ANOVA, RBD to test the statistical significance of variance among different treatment means as influenced with the application of the treatments on various attributes of maize.

Table 1. Characteristics of experimental soil at one soil depths (0-15 cm)

Particulars	Values
Soil pH (pH 1:2.5)	7.48
Organic carbon (%)	0.53
Available nitrogen (kg ha ⁻¹)	233
Available phosphorus (kg ha ⁻¹)	14.9
Available potassium (kg ha ⁻¹)	209
<i>P. fluorescens</i> population (cfu g ⁻¹ soil)	1.35×10 ⁴

2.1 Climate

The tropic of cancer passes through the middle of the district. It has sub-tropical climate characterized by hot dry summers and cool dry winter. Jabalpur lies in the "Kymore Plateau and Satpura hills" agro-climatic zone of Madhya Pradesh. Seasonal variations prevailing during the growing period plays an important role in the developmental process, which may have great influence on the final yield of the crop. The weekly meteorological data were recorded during crop season of Meteorological Observatory College of Agricultural Engineering, JNKVV, Jabalpur and are depicted through Fig. 1. It is obvious from the meteorological data that the weather conditions which prevailed during the crop season were favorable for the growth and development of maize. The mean weekly maximum temperature ranges from (26.2 to 44.4°C) whereas; mean weekly minimum temperature ranged between 3.1 to 27.3°C. Whereas, relative humidity varied from 49.0 to 85.0 % in morning and 17.0 to 47.0% in evening. The mean sunshine hours varied between 7.3 to 10.3 hours per day. Total rainfall of 77.3 mm was received.

2.2 Seed Inoculation, Treatment and Sowing

The isolates of *P. fluorescens* that performed best in the laboratory experiment (Component I) for population growth and ACCD enzyme activity were identified and particularly selected for the field trial on maize to see if the features were sustainable. However, remaining other isolates along with the selected isolates was also included in the field trial. The isolates in broth were used for seed treatment and foliar spray on maize at three growth stages (at knee stage, tasselling and silking stage) to ascertain the population growth attributes and ACCD activity in rhizosphere and the plant growth and yield at maturity. Maize seeds in polythene bags were slightly moistened and then treated with carbendazim fungicide 2 g kg⁻¹ seed and air dried under shade. Then the seeds were inoculated individually with the isolated of *P. fluorescens* the recommended dose 10 ml kg⁻¹ of seed and using sterilized gum acacia (2%) as adhesive. The field experiment was carried out at research farm JNKVV Jabalpur during winter season of 2019. The seeds of maize (cv., JM-216) were sown in the respective plot. Recommended package of practices (Depth of

Sowing: 4-6 cm, where maize is generally sown on flat beds. Spacing: 70cm x 25cm for row to row and plant to plant. Plant density of 66,666/ha and intercultural operation) was followed to maintain plant population, protection and growth.

2.3 Analysis of Soil Samples

The surface (0-15 cm) soil samples were collected from the experimental site at knee height, tasseling stage and silking stage of maize crop. The soil samples were air dried and crushed with wooden pestle and mortar and sieved through 2 mm sieve. The material passed through the sieve was used for determination of various characters. Available nitrogen in soil sample was determined by using alkaline permanganate method [9]. A known weight of soil is mixed with excess of alkaline permanganate and distilled. Organic matter present in soils is oxidized by the nascent oxygen liberated by KMnO₄ in the presence of NaOH and thus ammonia is released. This released ammonia was absorbed in the boric acid (2%) solution containing mixed indicator and converted to ammonium borate. This ammonium borate was titrated with standard sulphuric acid. The available phosphorus content in soil was estimated by extraction procedure as described by [10]. Soil available phosphorus was extracted using 0.5 M NaHCO₃ (pH 8.5) and determination was done by ascorbic acid method as described by [11]. The available potassium was extracted with neutral 1 N ammonium acetate as described by [12] and determined by Flame photometer.

3. RESULTS AND DISCUSSION

Results of field experiments revealed that inoculation with selected *P. fluorescens* isolates, containing ACCD activity, under field conditions significantly promoted the available nutrients in soil. However, the rate of enhancement varied with isolates obtained from component (I). Inoculation of rhizobacterial isolates in the presence of chemical fertilizer significantly increased available nutrients in soil of maize.

3.1 Available Nitrogen in Soil

The data regarding available nitrogen contents in surface soil (up to 0-15 cm depth) at knee height, tasseling and silking stage of the crop are presented in Table 2.

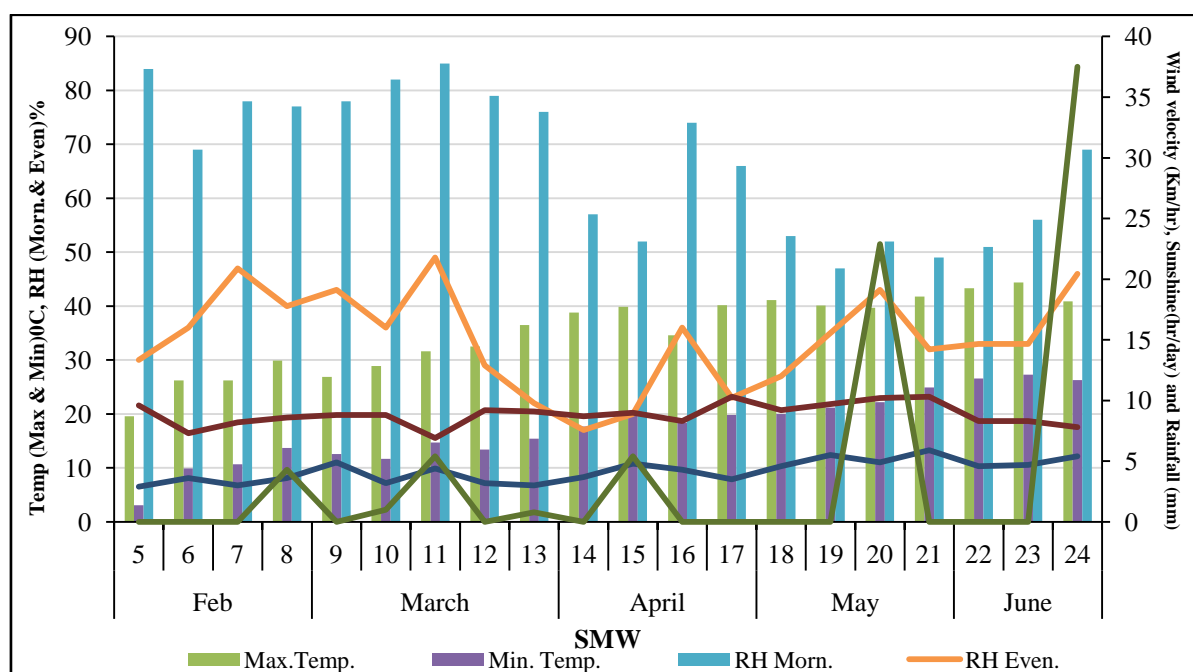


Fig. 1. Weekly meteorological data during Feb - Jun, 2018-19

Table 2. Effect of selected isolates of *P. fluorescens* on available nitrogen in soil of maize at different growth stages

Treatment combination	Available N (kg ha ⁻¹)		
	knee height	Tasseling stage	Silking stage
T ₂₅ +pH 6.7	237	212	189
T ₂₅ +pH 7.2	250	227	204
T ₂₅ +pH 7.7	245	220	197
T ₂₅ +pH 8.2	233	208	185
T ₂₈ +pH 6.7	241	218	193
T ₂₈ +pH 7.2	254	230	206
T ₂₈ +pH 7.7	246	223	200
T ₂₈ +pH 8.2	235	210	187
T ₃₁ +pH 6.7	240	215	192
T ₃₁ +pH 7.2	252	229	205
T ₃₁ +pH 7.7	246	221	198
T ₃₁ +pH 8.2	235	210	187
T ₃₄ +pH 6.7	238	213	190
T ₃₄ +pH 7.2	249	225	203
T ₃₄ +pH 7.7	230	205	182
T ₃₄ +pH 8.2	229	204	181
FUI	209	196	180
UFUI	194	179	166
Mean	237	214	191
SE _m ±	9.2	5.6	4.2
CD _{5%}	27.0	16.4	12.5

The available N content in soil at knee height stage varied from 194 to 254 kg N ha⁻¹ with the mean value of 237 kg N ha⁻¹. The isolates of T₂₈+pH 7.2 significantly increase the N content 254 kg N ha⁻¹ in soil with response of 21.5% over FUI (209 kg N ha⁻¹) followed by T₃₁+pH 7.2,

T₂₅+pH 7.2, T₃₄+pH 7.2, T₂₈+pH 7.7, T₃₁+pH 7.7, T₂₅+pH 7.7, T₂₈+pH 6.7, T₃₁+pH 6.7, T₃₄+pH 6.7 and T₂₅+pH 6.7 with N content in soil of 252, 250, 249, 246, 246, 245, 241, 240, 238 and 237 kg N ha⁻¹ by 20.6, 19.6, 19.1, 17.7, 17.7, 17.2, 15.3, 14.8, 13.9 and 13.4% response, respectively

over the FUI. The available N content in soil at tasseling stage increased from 179 to 230 kg N ha⁻¹ with mean value of 214 kg N ha⁻¹. On comparing all the treatments, the available N content of 230 kg N ha⁻¹ was found significantly superior by the application of T₂₈+pH 7.2 as compared to control FUI (196 kg N ha⁻¹) and it was responded by 17.3%, followed by the treatment combination T₃₁+pH 7.2, T₂₅+pH 7.2, T₃₄+pH 7.2, T₂₈+pH 7.7, T₃₁+pH 7.7, T₂₅+pH 7.7, T₂₈+pH 6.7, T₃₁+pH 6.7 and T₃₄+pH 6.7 with N content in soil 229, 227, 225, 223, 221, 220, 218, 217 and 213 kg N ha⁻¹ along with 16.8, 15.8, 14.8, 13.8, 12.8, 12.2, 11.2, 9.7 and 8.7% response, over FUI. The N content in soil at silking stage varied from 166 to 206 kg N ha⁻¹ with average value of 191 kg N ha⁻¹. Among the treatments, the available N content of 206 kg N ha⁻¹ in soil at surface layer was found statistically superior by the use of T₂₈+pH 7.2 as compared to FUI (166N kg ha⁻¹) and it was responded about 14.4% followed by T₃₁+pH 7.2, T₂₅+pH 7.2, T₃₄+pH 7.2, T₂₈+pH 7.7, T₃₁+pH 7.7, T₂₅+pH 7.7, T₂₈+pH 6.7 and T₃₁+pH 6.7 with 205, 204, 203, 200, 198, 197 and 193 kg N ha⁻¹ in soil at surface layer along with 13.9, 13.3, 12.8, 11.1, 10.0, 9.4 and 7.2% response, over FUI.

Rhizobial strains that express ACC deaminase are up to 40% more efficient at forming nitrogen-

fixing than strains that lack this activity. The slight buildup of the soil available N could be attributed to increased activity of nitrogen fixing and phosphate solubilizing rhizobacteria thereby resulting in higher accumulation of N in the soil [13]. Levels of these were increased over a period of time due to their residual or cumulative effect through addition of these nutrients [14,15,16]. Also observed increased available N content in soil after the harvest of the crop due application of microbial inoculants as compared to no inoculation.

3.2 Available Phosphorus in Soil

The data regarding available P contents in surface soil (up to 0-15 cm depth) at knee height, tasseling and silking stage of the crop are presented in Table 3.

The available P content in soil at knee height stage varied from 16.8 to 23.7 kg P ha⁻¹ with the mean value of 21.7 kg P ha⁻¹. The isolates of T₂₈+pH 7.2 significantly increase the P content (23.7 kg P ha⁻¹) in soil with response of 31.7% over FUI (18.0 kg P ha⁻¹) followed by T₃₁+pH 7.2, T₂₅+pH 7.2, T₃₄+pH 7.2, T₂₈+pH 7.7 and T₃₁+pH 7.7 with P content in soil of 23.0, 22.8, 22.6, 22.5 and kg P ha⁻¹ by 27.8, 26.7, 25.6, 25.0 and 24.4% response, respectively over the FUI. The

Table 3. Effect of selected isolates of *P. fluorescens* on available phosphorus in soil of maize at different growth stages

Treatment combination	Available P (kg ha ⁻¹)		
	knee height	Tasseling stage	Silking stage
T ₂₅ +pH 6.7	20.5	17.7	15.7
T ₂₅ +pH 7.2	22.8	19.5	17.5
T ₂₅ +pH 7.7	21.6	18.7	16.7
T ₂₅ +pH 8.2	19.2	17.2	15.2
T ₂₈ +pH 6.7	21.3	18.4	16.4
T ₂₈ +pH 7.2	23.7	20.2	18.2
T ₂₈ +pH 7.7	22.5	19.0	17.0
T ₂₈ +pH 8.2	19.9	17.5	15.5
T ₃₁ +pH 6.7	21.1	17.8	15.8
T ₃₁ +pH 7.2	23.0	19.7	17.7
T ₃₁ +pH 7.7	22.4	19.0	17.0
T ₃₁ +pH 8.2	19.6	17.2	15.2
T ₃₄ +pH 6.7	20.8	18.0	16.0
T ₃₄ +pH 7.2	22.6	19.2	17.2
T ₃₄ +pH 7.7	18.9	16.9	14.9
T ₃₄ +pH 8.2	18.5	16.7	14.7
FUI	18.0	16.4	14.4
UFUI	16.8	15.5	13.8
Mean	20.7	18.0	16.1
SE _{m±}	1.3	0.8	0.8
CD _{5%}	3.8	2.3	2.5

available P content in soil at tasseling stage increased from 15.5 to 20.2 kg P ha⁻¹ with mean value of 18.0 kg P ha⁻¹. On comparing all the treatments, the available phosphorous content of 20.2 kg P ha⁻¹ was found significantly superior by the application of T₂₅+pH 7.2 as compared to control FUI (16.4 kg P ha⁻¹) and it was responded by 23.2%, followed by the treatment combination T₃₁+pH 7.2, T₂₅+pH 7.2, T₃₄+pH 7.2, T₂₈+pH 7.7 and T₃₁+pH 7.7 with P content in soil 19.7, 19.5, 19.2, 19.0 and 19.0 kg P ha⁻¹ along with 20.1, 18.9, 17.1, 15.9 and 15.9% response, over FUI. The available P content at silking stage varied from 13.8 to 18.2 kg P ha⁻¹ with average value of 16.1 kg P ha⁻¹. Among the treatments, the available P content of 18.2 kg P ha⁻¹ in soil at surface layer was found statistically superior by the use of T₂₈+pH 7.2 as compared to FUI (14.4 kg P ha⁻¹) and it was responded about 26.4%, followed by T₃₁+pH 7.2, T₂₅+pH 7.2, T₃₄+pH 7.2, T₂₈+pH 7.7 and T₃₁+pH 7.7 with 17.7, 17.5, 17.2, 17.0 and 17.0 kg P ha⁻¹ in soil at surface layer along with 22.9, 21.5, 19.4, 18.1 and 18.1% response, over FUI.

The availability of P status of soil improved due to PGPR may be ascribed to increased solubility of unavailable native soil phosphate with exotic strains of PSB besides extraction of P from deeper layers by VAM hyphae [16]. Results were

also in accordance with the findings of [15,2,17,18,19,20] that the result revealed that application of fertilizer levels, farm yard manure (FYM) and bioinoculants on soil properties in inceptisol of varanasi, uttar pradesh, india. The integration of inorganic fertilisers with FYM and bioinoculants significantly improved the available N, P, and K as well as the microbial population of soil after wheat harvest. The application of biological and organic manure has had a positive impact, with the added benefit of reducing the use of chemical fertiliser.

3.3 Available Potassium in Soil

The data regarding available potassium contents in surface soil (up to 0-15 cm depth) at knee height, tasseling and silking stage of the crop are presented in Table 4.

The available K content in soil at knee height stage varied from 273 to 341 kg K ha⁻¹ with the mean value of 326 kg K ha⁻¹. The isolates of T₂₈+pH 7.2 significantly increase the K content 341 kg K ha⁻¹ in soil with response 14.4% over FUI (298 kg K ha⁻¹), followed by T₃₁+pH 7.2, T₂₅+pH 7.2, T₃₄+pH 7.2, T₂₈+pH 7.7, T₃₁+pH 7.7, T₂₅+pH 7.7 and T₂₈+pH 6.7 with K content in soil of 338, 337, 336, 335, 334, 332, 332 and

Table 4. Effect of selected isolates of *P. fluorescens* on available potassium in soil of maize at different growth stages

Treatment combination	Available K (kg ha ⁻¹)		
	knee height	Tasseling stage	Silking stage
T ₂₅ +pH 6.7	327	295	279
T ₂₅ +pH 7.2	337	303	288
T ₂₅ +pH 7.7	332	299	283
T ₂₅ +pH 8.2	323	293	276
T ₂₈ +pH 6.7	332	298	283
T ₂₈ +pH 7.2	341	306	291
T ₂₈ +pH 7.7	335	301	287
T ₂₈ +pH 8.2	326	295	278
T ₃₁ +pH 6.7	330	297	281
T ₃₁ +pH 7.2	338	305	289
T ₃₁ +pH 7.7	334	299	285
T ₃₁ +pH 8.2	325	294	277
T ₃₄ +pH 6.7	328	296	280
T ₃₄ +pH 7.2	336	302	282
T ₃₄ +pH 7.7	322	292	276
T ₃₄ +pH 8.2	322	291	275
FUI	298	278	264
UFUI	273	267	255
Mean	326	295	279
SE _m ±	10.3	6.6	5.6
CD _{5%}	30.2	19.4	16.5

330 kg K ha⁻¹ by 13.4, 13.1, 12.8, 12.1, 11.4, 11.4 and 10.7% response, respectively over the FUI. The available K content in soil at tasseling stage increased from 267 to 306 kg K ha⁻¹ with mean value of 295 kg K ha⁻¹. On comparing all the treatments, the available K content of 306 kg K ha⁻¹ was found significantly superior by the application of T₂₈+pH 7.2 as compared to control FUI (278 kg K ha⁻¹) and it was responded by 10.1%, followed by the treatment combination T₃₁+pH 7.2, T₂₅+pH 7.2, T₃₄+pH 7.2, T₂₈+pH 7.7, T₃₁+pH 7.7, T₂₅+pH 7.7 and T₂₈+pH 6.7 with K content in soil 305, 303, 302, 301, 299, 299 and 298 kg K ha⁻¹ along with 9.7, 9.0, 8.6, 8.3, 7.6, 7.6 and 7.2% response, over FUI. The K in soil at silking stage varied from 255 to 291 kg K ha⁻¹ with average value of 279 kg K ha⁻¹. Among the treatments, the available K content of 291 kg K ha⁻¹ in soil at surface layer was found statistically superior by the use of T₂₈+pH 7.2 as compared to FUI (264 kg K ha⁻¹) and it was responded about 10.4% followed by T₃₁+pH 7.2, T₂₅+pH 7.2, T₃₄+pH 7.2, T₂₈+pH 7.7, T₃₁+pH 7.7, T₂₅+pH 7.7, T₂₈+pH 6.7 and T₃₁+pH 6.7 with 289, 288, 282, 287, 285, 283, 283 and 281 kg K ha⁻¹ in soil at surface layer along with 9.6, 9.2, 7.0, 8.8, 8.1, 7.3, 7.3 and 6.6% response, over FUI.

The beneficial effect of PGPR on K availability includes minimizing the losses due to fixation as well as release of K through the action of organic acids liberated during decomposition. The results were in accordance with the findings of [16,21,22] also reported that combined inoculation of Rhizobium, (phosphorous solubilizing Bacteria) PSB and PGRR improved the nutrient status of soil through their synergistic effect on nitrogen fixation and solubilisation of native soil phosphorus which increased availability these nutrients by the crop plant (Urd bean) resulted in enhanced yield indices [23,24]. Seed inoculation with biofertilizers enhanced available N, P, K contents of the soil.

4. CONCLUSION

In conclusion, ACC deaminase-containing bacteria might prove beneficial in agriculture towards crop production and conservation and the isolates are likely to be potential candidates for biofertilizers, biocontrol and biotechnological application of commercial value [25]. The use of PGPR containing ACC deaminase activity along with other innovations could prove to be an environmentally friendly strategy to ensure sustainable agriculture. Further work is needed to evaluate the effectiveness of these ACC

deaminase-containing bacterial strains under field conditions.

ACKNOWLEDGEMENTS

The authors would like to thank the Microbes Research and Production Centre, Department of Soil Science and Agricultural Chemistry, J.N. Krishi Vishwa Vidyalaya, Jabalpur, for providing necessary facility during research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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