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Relative efficacy of *Pseudomonas fluorescens* containing ACC-deaminase for improving nutrient content, soil fertility and yield of maize

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Abstract
The present investigation was carried out at JNKVV, Jabalpur during summer season of 2019 to evaluate the performance of previously multiply of *Pseudomonas* (P) *fluorescens* at different temperature and pH value on nutrient content and their uptake by plant, nutrient availability and yield of maize (*Zea mays* L.). Treatments comprised of 16 isolated from fermentor under different temperature and pH (Temperature: 25, 28, 31, 34 ^{0}C and pH 6.7, 7.2, 7.7, 8.2) including two control FUI (fertilized + uninoculated) and UFUI (unfertilized + uninoculated). Results indicated that inoculation with different isolates had positive impact on total NPK content and their uptake by crop, NPK availability and yield of maize (grain and stover) comparing to FUI. The treatment combination T_{28}+pH 7.2 (Temp 28 ^{o}C + pH 7.2) isolates significantly enhanced the grain and stover N (12.8 and 8.8%) and P (17 and 10.3%) content respectively, and the isolate of T_{31}+pH 7.2 significantly increased K content in grain and stover (26.7 and 26.5%) respectively, over that of FUI. The treatment combination T_{28}+pH 7.2 isolates significantly enhanced the total N and K uptake by plant (42.2 and 45.8%) respectively over that of FUI and the isolate of T_{31}+pH 7.2 significantly increased total P content by plant (61.1%) over that of FUI. The treatment combination T_{28}+pH 7.2 isolates significantly improvement in available N (16.0%), P (25.7%) and K (25.2%) content in soil after harvest of crop over that of FUI. The treatment combination T_{28} + pH 7.2 significantly increased the grain (24.9%) and stover yields (31.6%) of maize over that of FUI. This study demonstrates the application of *P. fluorescens* play a vital role for improving the NPK contents their uptake, nutrient availability and yield of maize.

Keywords: *Pseudomonas fluorescens,* soil fertility, maize yield and nutrient uptake

1. Introduction
Maize (*Zea mays* L) is called king of cereal because of it productivity potential compared to any other cereal crop (Umesha *et al.*, 2014) [24]. The cultivation of maize spread rapidly around the globe and currently it is being produced in most countries of the world. Maize plant is a major source of food for both human and animals, and is grown in more countries than other crop. Maize can only be produced in areas that do not have extreme cold temperature. The majority of the crop is used as livestock feed; the remainder is processed into a range of food and industrial products including starch, ethanol for use as fuel sweetness such as high fructose maize syrup and maize oil. In India, maize is grown in an area of 7.18 million ha contributing 14.1 million tonnes of production with a productivity of 1959 kg ha^{-1}. The state of Madhya Pradesh occupies 13% of the total maize area and contributing equally to the total maize production in the India. Nutritionally, maize contains 60-68% starch, 1.2-5.7% edible oil and 7- 15% protein. (Agriculture Statistics at a glance, 2016)

Introduction of plant growth promoting rhizobacteria (PGPR) including *P. fluorescens* as biofertilizers is suggested as a sustainable option for the improvement of nutrient availability, plant growth and yields (Vessey, 2003) [25]. Use of microbial consortia in the form of biofertilizers for reducing the use of chemical fertilizers without compromising yield is presently an important feature of research in the field of agriculture, micro- biology and biotechnology (Minorsky, 2008) [15]. The search for diverse PGPRs is gaining serious attention and efforts are made to exploit them as biofertilizers for various economically important crops. During the last two decades use of microbial techniques and introduction of rhizobacteria in agriculture has increased tremendously due to their potential for N_2-fixation and P solubilization thus increasing N and P uptake by the plants and therefore yields (Vessey, 2003) [25]. Successful results of using PGPR species including *Azospirillum, Bacillus*, *Pseudomonas* and *Enterobacteria* on maize, canola, wheat, and other horticultural crops have been achieved both in the laboratory and in the field under variable ecological conditions (Mehta *et al*., 2014) [13].

Treatment of plant seeds and seedlings with PGPR can either directly or indirectly enhance the growth of plants (Glick, 1995) [6]. In direct growth stimulation, these bacteria provide soluble phosphate, fix nitrogen, deaminase and phytohormones like indole acetic acid (IAA) and increase the bioavailability of iron through bacterial siderophore for plants (Lucy *et al*., 2004; Glick *et al*., 2007) [12, 5]. Indirect promotion of growth occurs when these bacteria protect plants from detrimental effects of plant pathogens, e.g.by competition, antibiosis and hyperparasitism (Raaijmakers *et al*., 2012) [18]. PGPRs are also capable of increasing plant resistance to biotic and abiotic environmental stresses. One of the main mechanisms by which rhizobacteria exert positive effects on plants under abiotic stresses is production of ACC deaminase and regulation of ACC, a precursor to stress-induced ethylene in host plants (Glick *et al*., 1998) [4]. The effectiveness of rhizobacteria with ACC deaminase activity has been reported in reducing stress ethylene, conferring beneficial effects to plants under various environmental stressors such as pathogen and insect infection, high salinity, drought and heavy metal contamination (Yildirim *et al*., 2006) [27].

The present study is focused on relative efficacy of *P. fluorescens* containing ACC-deaminase for improving soil fertility and yield of maize. This approach could help to obtain high yield potential and also reduce dependence on chemical fertilizers without compromising per unit yield of maize.

2. Materials and methods

The experiment was carried out at research field, Department of Soil Science & Agricultural Chemistry, JNKVV, Jabalpur during summer season of 2019 to evaluate the performance of previously multiply (in 2018) of *Pseudomonas* (P) *fluorescens* at different temperature (25, 28, 31, 34 ^{0}C) and pH (6.7, 7.2, 7.7, 8.2) value with treatments comprised of 16 isolated from fermenter including two control FUI (fertilized + uninoculated) and UFUI (unfertilized + uninoculated). The experiment was laid out in randomized block design with 3 replications. The strain of *P. fluorescens* was obtained from the project AINP on Soil Biodiversity & Biofertilizers (ICAR), JNKVV, Jabalpur. All the technical efforts were endeavored to maintain the population up to the standard 10^{-8} to 10^{-9} cfu ml^{-1}. The selected isolated was directly used for the experiment and recommended dose of fertilizer 120:60:60N:P_2O_5:K_2O kg ha^{-1} for maize crop in the form of urea, single superphosphate and muriate of potash, respectively. N and K were supplemented as basal application to each plot as per recommendation and P was applied as per scheduled dose of treatments.

The isolates of *P. fluorescens* obtained from the laboratory experiment (Component I) performing best for population growth and ACC-deaminase enzyme activity were earmarked and specially selected for the field trial on maize to observe sustainability of the attributes. 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 hight, tesseling and silking stage).

Maize seeds in polythene bags were slightly moistened and then treated with carbendazim fungicide @ 2 g kg-1 seed. Seeds were allowed to air dry under shade. Then the seeds were inoculated individually with the isolated of *P. fluorescens* bioinoculant at double the recommended dose 20 ml or g 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 (JM-216) were sown in the respective plot. Recommended package of practices was followed to maintain plant population, protection and growth.

During this study, NPK content and their uptake by plant, crop yield and soil nutrient availability were measured, on each plot in the considered experimental site. The surface (0-15 cm depth) soil samples were collected from the experimental site before sowing of crop and after harvest. 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. N content was determined by Kjeldahl method and P was determined in digest (HNO_3: $HCLO_4$) by vanadomolybdate yellow colourmethod (Jackon 1973) and the K content was estimated from acid digest (3.10.2) with a flame photometer using the procedure of Bhargava and Raghupathi (1984) [2]. Uptake of the NPK was calculated by multiplying yield with content of nutrients in grain and stover. Available N in soil was determined by using alkaline permanganate method (Subbiah and Asija, 1956) [22]. The P content of soil was estimated by extraction procedure as described by Olsen *et al.*, (1954) [17]. Soil available P was extracted using 0.5 M $NaHCO_3$ (pH 8.5) and determination was done by ascorbic acid method as described by Miller and Keeney (1982). The available K was extracted by neutral 1N ammonium acetate and it was estimated using flame photometer (Mohr *et al.*, 1963) [16]. The crop was harvested plot wise and yields of seed and stover were recorded.

3. Results and discussion

Results of field experiments revealed that inoculation with *P. fluorescens* isolates, containing ACC-deaminase activity, under field conditions significantly improvement the nutrient contents their uptake, soil fertility including available N, P and K and yield of maize.

3.1 NPK content in maize

The quality of any produce is judged by the content of nutrients in the economic produce. Further, the use efficiency of the applied inputs is related to total utilization and uptake by the growing crop.

The data on N content in grain varied from 1.27-1.47% N, respectively with the average value 1.37% N. Among all the treatments, the T_{28}+pH 7.2 isolates were recorded maximum N content of 1.47% N. It was interesting to note that the percent increment were computed 12.8%, by grain, respectively over FUI of grain (1.30% N). The data pertaining to the P content in grain varied from 0.32 to 0.44% P, respectively with the mean value 0.38% P. On comparing all the treatments, the T_{31}+pH 7.2 isolates were recorded maximum P content of 0.44% P grain in maize. It was interesting to note that the percent increment were computed 26.7% by grain, respectively over FUI (0.35% P). The data related to the K contents in grain increased from 0.56- 0.71% K with the mean values of 0.65% K. The isolates of T_{28}+pH 7.2 were recorded statistically higher for K content of 0.71% K by seed of maize with percent response of 17.0%

respectively over FUI (0.61% K). This was followed by the treatment combinations of T_{31}+pH 7.2 and T_{25}+pH 7.2 by grain of 0.70 and 0.70% K with response of 15.4 and 15.3% by grain, respectively (table 1).

The data on N content in stover varied from 0.95-1.07% N, respectively with the average value 1.02% N. Among all the treatments, the T_{28}+pH 7.2 isolates were recorded maximum N content of 1.07% N. It was interesting to note that the percent increment were computed 8.8%, respectively over FUI (0.95% N). The data pertaining to the P content in stover varied from 0.28-0.35% P, respectively with the mean value 0.31% P. On comparing all the treatments, the T_{31}+pH 7.2 isolates were recorded maximum P content of 0.35% P. It was interesting to note that the percent increment were computed 26.5% by stover, respectively over FUI (0.28% P). The data related to the K contents in stover increased from 0.83 to0.93% K with the mean values of 0.88% K. The isolate of T_{28}+pH 7.2 was recorded statistically higher for K content of 0.93% K by stover of maize with percent response of 10.3%, respectively over FUI (0.89% K). This was followed by the treatment combinations of T_{31}+pH 7.2, T_{25}+pH 7.2, T_{34}+pH 7.2, T_{28}+pH 7.7 and T_{31}+pH 7.7 by stover of 0.92, 0.92, 0.91, 0.91 and 0.90% K with response of 9.5, 9.5, 8.3, 7.9 and 7.1% by stover, respectively (Table 1).

This is reflective of the efficiency of the microorganisms in promoting plant growth, nutrient content, biomass and thereby yields due to effective nutrient mobilization from soil to plant. Our earlier publications (Joshi *et al.*, 2007) [7] have illustrated a similar trend. It can therefore be surmised that the PGP traits of the micro-organisms used and their colonization in the rhizosphere lead to the enhanced yields and nutrient quality of grains. Rana *et al*., (2012) [19] the combined inoculation of *Anabaena oscillarioides* CR3, *Brevundimonas Diminuta* PR 7, and *Ochrobactrum anthropic* PR10 (T6) significantly increased NPK content and improved rice yield by 21.2%, as compared to the application of recommended dose of NPK fertilizers.

Table 1: Influence of isolates of *P. fluorescens* obtained from fermentation study at different temperature and pH on NPK contents in grain and stover of maize

Treatment combination	Nutrient content (%)					
	Grain			Stover		
	N	P	K	N	P	K
T_{25}+pH 6.7	1.35	0.36	0.65	1.01	0.29	0.87
T_{25}+pH 7.2	1.43	0.42	0.69	1.05	0.34	0.92
T_{25}+pH 7.7	1.40	0.39	0.67	1.03	0.32	0.90
T_{25}+pH 8.2	1.33	0.35	0.63	1.00	0.29	0.86
T_{28}+pH 6.7	1.38	0.38	0.67	1.03	0.31	0.89
T_{28}+pH 7.2	1.47	0.43	0.71	1.07	0.34	0.93
T_{28}+pH 7.7	1.41	0.41	0.68	1.05	0.33	0.91
T_{28}+pH 8.2	1.34	0.39	0.65	1.01	0.29	0.87
T_{31}+pH 6.7	1.37	0.37	0.66	1.02	0.31	0.89
T_{31}+pH 7.2	1.45	0.44	0.70	1.06	0.35	0.92
T_{31}+pH 7.7	1.40	0.40	0.68	1.04	0.32	0.90
T_{31}+pH 8.2	1.34	0.36	0.64	1.00	0.28	0.86
T_{34}+pH 6.7	1.36	0.37	0.66	1.02	0.30	0.88
T_{34}+pH 7.2	1.42	0.41	0.69	1.05	0.33	0.91
T_{34}+pH 7.7	1.32	0.36	0.62	1.00	0.29	0.85
T_{34}+pH 8.2	1.32	0.35	0.61	1.00	0.29	0.85
FUI	1.30	0.35	0.61	0.99	0.28	0.84
UFUI	1.27	0.32	0.56	0.95	0.26	0.83
Mean	1.37	0.38	0.65	1.02	0.31	0.88
SE_m±	0.03	0.02	0.03	0.02	0.01	0.02
$CD_{5\%}$	0.09	0.05	0.09	0.06	0.04	0.06

3.2 NPK uptake by maize

The N, P and K uptake by crop was presented in table 2. The data on total N uptake by plant ranged from 87-154 kg N ha^{-1}, respectively with the average value 125 kg N ha^{-1}. Among all the treatments, the T_{28}+pH 7.2 isolates were recorded higher N uptake of 154 kg N ha^{-1}. It was interesting to note that the percent increment were computed 42.2%, respectively over FUI (108 kg N ha^{-1}). The data on total P uptake by plant ranged from 23.1-48.1 kg P ha^{-1}, respectively with the average value 36.8 kg P ha^{-1}. Among all the treatments, the T_{31}+pH 7.2 isolates were recorded maximum P uptake of 48.1 kg P ha^{-1}. It was interesting to note that the percent increment were computed 61.1%, respectively over FUI (30.0 kg P ha^{-1}). The data on total K uptake by plant ranged from 62 to 113 kg K ha^{-1}, respectively with the average value 91 kg K ha^{-1}. Among all the treatments, the T_{28}+pH 7.2 isolates were recorded higher uptake of 113 kg K ha^{-1}. It was interesting to note that the percent increment were computed 45.8%, respectively over FUI (78 kg K ha^{-1}).

The possible reason might be associated with the initial increase in root growth by the application of PGPR strains, which could promote better absorption of essential nutrients. PGPR synthesize phytohormones that promote plant growth at various stages (Kloepper *et al.*, 1986) [11]. Similarly finding was Vikram (2007) [26] reported that inoculation with PSB V-l recorded, the highest ear head weight, shoot length, shoot dry matter, grain yield, P content and P uptake in root and grain of sorghum plants. The high increase in P uptake and its consequent reflection on yield as an effect of PSB inoculation may be caused by the ability of PSB strains to solubilize insoluble inorganic phosphates as well as to produce necessary phytohormones (Hameeda *et al.*, 2006) [7]. All PSB strains used in the present study produced considerable amounts of IAA and GA besides being efficient in solubilization of insoluble inorganic phosphates. Supanjani *et al.* (2006) [23] showed that the co-inoculation of potassium and PSB increased P availability from 12-21% and K availability from 13-15% and also subsequently improved N, P and K uptake. The integration also increased the biomass harvest and yield of the treated plants were increased by 23-30% which showed that it was sustainable alternative to the use of chemical fertilizer.

Table 2: Effect of isolates of *P. fluorescens* obtained from fermentation study at different temperature and pH on total NPK uptake and yield of Maize (grain and stover)

Treatment combination	Total nutrient uptake (kg ha^{-1})			Yield (kg ha^{-1})	
	N	P	K	Grain	Stover
T_{25}+pH 6.7	117	32.8	85	3033	7502
T_{25}+pH 7.2	148	46.0	109	3552	9205
T_{25}+pH 7.7	132	39.3	97	3353	8257
T_{25}+pH 8.2	112	31.3	81	2950	7247
T_{28}+pH 6.7	129	37.5	94	3319	8037
T_{28}+pH 7.2	154	47.6	113	3641	9387
T_{28}+pH 7.7	142	43.5	104	3452	8900
T_{28}+pH 8.2	116	33.6	85	2999	7485
T_{31}+pH 6.7	125	36.3	91	3235	7855
T_{31}+pH 7.2	151	48.3	110	3590	9248
T_{31}+pH 7.7	134	40.3	98	3427	8305
T_{31}+pH 8.2	113	31.6	82	2977	7357
T_{34}+pH 6.7	122	35.0	89	3182	7756
T_{34}+pH 7.2	144	43.9	106	3478	8964
T_{34}+pH 7.7	110	31.1	79	2918	7150
T_{34}+pH 8.2	110	31.1	78	2935	7140
FUI	108	30.0	78	2914	7133
UFUI	87	23.1	63	2440	5874
Mean	125	36.8	91	3189	7934
$SE_m\pm$	4.2	1.5	3.3	142.5	233.9
$CD_{5\%}$	12.3	4.4	9.6	420.0	689.3

3.3 Grain and stover yields

The data related to the grain and stover yield of the maize crop is given in table 2. The grain yield of maize ranged from 2440 to 3641 kg ha^{-1} with the average value of 3189 kg ha^{-1}. The grain yields of maize due to every treatment under study differed significantly over the control of FUI. Among all the treatments, the treatment combination of T_{28}+pH 7.2 yielded significantly maximum grain yield of 3641kg ha^{-1} that was responded 24.9% over FUI (2914 kg ha^{-1}).

The stover yield of maize increased from 5874 to 9387 kg ha^{-1} with the mean value of 7934 kg ha^{-1}. The highest stover yield of maize 9387 kg ha^{-1} was recorded with the treatment combination of T_{28}+pH 7.2 by 31.6% response over the control FUI (7133 kg ha^{-1}).

Gholami *et al.* (2009) [3] studied the effect of PGPR on seed germination, seedling growth and yield of field grown maize. The results showed that inoculation with bacterial treatments had a more stimulating effect on growth and development of plants. Inoculation of maize seeds with bacterial strains significantly increased plant height, 100 seed weight, number of seed per ear and yield of maize. Such an improvement might be attributed to N_2-fixing and phosphate solubilizing capacity of bacteria as well as the ability of these microorganisms to produce growth promoting substances (Salantur *et al.*, 2006) [20].

On the basis of findings, it may be concluded that the application of *P. fluorescens* isolates of T_{28}+pH 7.2 obtained from fermenter under different condition of pH and temperature. The application of ACC-deaminase containing PGPR improving nutrient content their uptake by crop, soil physiochemical properties and yield of maize.

3.4 Available N, P and K in soil after harvest

Table 3 and depicted data on available N, P and Kin soil after harvest of the crop. The available N content in soil at harvest of the crop varied from 154-195 kg ha^{-1} with the mean value of 179 kg ha^{-1}. The isolates of T_{28}+pH 7.2 significantly increase the N content 195 kg ha^{-1} in soil with response of 16.0% over FUI (168 kg ha^{1}). The available P content in soil at harvest of the crop varied from 11.7-15.2 kg P ha^{-1} with the mean value of 13.2 kg P ha^{-1}. The isolates of T_{28}+pH 7.2 significantly increase the P content (15.2 kg P ha^{-1}) in soil with response of 25.7% over FUI (12.1 kg ha^{-1}). The available K content in soil at harvest of the crop varied from 237-273 kg ha^{-1} with the mean value of 257 kg ha^{-1}.The isolates of T_{28}+pH 7.2 significantly increase the K content 273 kg ha^{-1} in soil with response of 25.2% over FUI (241 kg ha^{-1}).

Parewa *et al.*, (2014) results revealed that application of fertilizer levels, FYM and bioinoculant on soil properties in Inceptisol of Varanasi, Uttar Pradesh, India. The available N, P and K and microbial population of soil after the harvest of wheat were improved significantly due to the integration of inorganic fertilizers with FYM and bioinoculant. Positive impact of biological and organic manure application have been recorded with an additional advantage of reduction of chemical fertilizer use. Similarly findings was reported by Jakhar *et al.*, (2018) [21] Hayat *et al.*, (2010) [8] concluded that the inoculation of seed with PGPR or biofertilizers increase the mobility and availability of plant nutrients N, P and K in the soil.

Table 3: Influence of isolates of *P. fluorescens* obtained from fermentation study at different temperature and pH on available NPK in soil after harvest the crop

Treatment combination	Available nutrients (kg ha^{-1})		
	N	P	K
T_{25}+pH 6.7	177	12.7	257
T_{25}+pH 7.2	192	14.5	269
T_{25}+pH 7.7	185	13.7	263
T_{25}+pH 8.2	173	12.2	246
T_{28}+pH 6.7	181	13.4	261
T_{28}+pH 7.2	195	15.2	273
T_{28}+pH 7.7	188	14.0	266
T_{28}+pH 8.2	175	12.5	254
T_{31}+pH 6.7	180	12.8	259
T_{31}+pH 7.2	194	14.7	271
T_{31}+pH 7.7	186	14.0	264
T_{31}+pH 8.2	175	12.2	250
T_{34}+pH 6.7	178	13.0	258
T_{34}+pH 7.2	191	14.2	268
T_{34}+pH 7.7	170	12.5	245
T_{34}+pH 8.2	169	12.5	243
FUI	168	12.1	241
UFUI	154	11.7	237
Mean	179	13.2	257
$SE_m\pm$	4.8	0.5	5.6
$CD_{5\%}$	14.0	1.4	16.6

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