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Research Article

INOCULATION EFFECT OF EFFICIENT BACTERIAL K-ISOLATES AND LEVELS OF MINERAL K ON ENZYMATIC ACTIVITY IN RHIZOSPHERE OF MAIZE (*Zea mays* L) AT DIFFERENT GROWTH PERIODS

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Abstract- Soil microorganisms constitute the largest section of living things with varying potential in its habitat, biochemical, physiological processes and nutritional characteristics. They play an important role in remediation, biomedicine, environmental and agricultural area. Current trends in agriculture are focused on reduction of the use of chemical fertilizers and use of alternative ways to improve crop yield in a sustainable manner. JHK2 showed the maximum activity with 60 kg K_2O ha^{-1} and it was at par with the activity of BRG 6. The experimental treatments had a significant impact on rhizosphere soil enzyme activity. When K was applied @ 60 kg K_2O ha^{-1}, the urease activity of JHK2 and JHG11 was at par to each other. Application of 40 kg K_2O ha^{-1} of K, APN7 and RJJ4 resulted similar activities. Increasing the level from 20 to 60 kg K_2O ha^{-1}, RJJ 4 was found maximum activity. When K was applied @ 40 kg K_2O ha^{-1}, RJJ 4 and JHG 11 showed similar activity and 14.63% higher activity than control. In the entire crop period, the alkaline phosphatase activity increased initially at 30 DAS and then declined with the age of the crop.

Keywords- Efficient bacterial isolates, Enzymatic activity, Rhizosphere, maize, Bacterial K-isolates

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Introduction

Potassium is third most important essential element for plant growth after nitrogen and phosphorous. It is the seventh most abundant element in earth's crust. Potassium plays an important role in plant growth, performs many functions in plants like water movement, electrical balance, and regulation of hormones, opening and closing of stomata, resistance against biotic and abiotic stresses [1]. In soil, K exists mainly in four different pools: mineral, non-exchangeable, exchangeable and solution K. The concentration of soluble K in soils is meagre and its major portion (98%) exists as insoluble minerals [2, 3]. For optimum crop production, soil solution and exchangeable K need to be replenished continually with non-exchangeable K through weathering of K bearing minerals such as waste micas or by addition of K fertilizers. Plants can take potassium only from the soil solution. Its availability is dependent upon the K dynamics as well as on total K content of soil [4, 5].

Microorganisms play a key role in conversion of unavailable form of K, i.e. mineral form of potassium (muscovite, biotite, feldspar, orthoclase and illite) to available form i.e. solution form of potassium. These transformations have been a subject of study for a long time and still a matter of curiosity. Soil microorganisms and their enzymatic activities are the potential bio-indicator to assess the soil health so the use of these in existing situation can sustain the system a long run [6-8]. The activities of enzymes in the rhizosphere of plant are helped in decomposition of organic matter, nutrient cycling, enhancing soil fertility and crop productivity. Most of the microorganisms especially potassium solubilizing microorganisms release polysaccharide that help in growth of extracellular enzymes for the degradation of polymers that can also helped directly in preventing plant pathogenic fungi. Root exudate helps to sustain the soil microorganisms and rhizospheric enzymatic activity. Keeping these points in view comparison a study on effect of bacterial K-isolates and their effect on enzymatic activity of maize rhizosphere in order to reveal relationship between microbial diversity and enzyme activity in the rhizosphere.

Materials and Methods

A pot culture experiment was conducted at Institute of Agricultural Sciences (IAS), BHU, Varanasi during Kharif season of 2015. Soil for the pot experiment was collected from the field of IAS, BHU. The collected soil was air dried and ground, and passed through a 2 mm sieve. The experimental soil belongs to order Inceptisols. Four levels (0, 20, 40 and 60 kg K_2O ha^{-1}) of potassium applied during the pot filling as biotite and thoroughly mixed in soil. Maize crop variety Kanchan were grown and replicated thrice. Recommended dose of N, P_2O_5 and (kg ha^{-1}) were applied i.e. 100:60 for maize through urea and DAP. Half dose (50 %) of N and full doses of P_2O_5 and K_2O were applied as basal at the time of sowing in crop. The remaining N was applied as two equal splits at 30 and 60 DAS. Rhizospheric soil samples were taken by pull out the maize plant from pots with the help of Khurapi and collect the soil adhere to maize roots in polythene bag at 30 DAS, 60 DAS and at harvest.

Dehydrogenase activity

During respiration, biological oxidation of reduced compounds occurs which is catalyzed by dehydrogenases. During this process energy is evolved. Dehydrogenase activity was determined by Triphenyl Tetrazolium Chloride (TTC) method [9]. Air dried soil samples (6 g) were incubated with 1 ml of 3% TTC and 2.5 ml of water at 37°C for 24 hours. The amount of Triphenyl Tetrazolium Formazan (TTF) formed after the incubation period was extracted with methanol and quantified spectro-photometrically at 485 nm wavelength using standard TTF. The dehydrogenases can be assayed and activity can be expressed as the rate of formation of TPF from TTC. Higher the biological activity faster was the formation

of TPF [10].

Phosphatase activity

Colorimetric estimation of the p-nitrophenol released by phosphor-monoesterase activity, when the soil is incubated with buffer at pH 11 alkaline phosphor-monoesterase activities, sodium p–nitrophenyl phosphate solution and toluene. Alkaline phenol has a yellow colour, allowing it to be estimated colourimetrically. The $CaCl_2$ – NaOH treatment described for extraction of p-nitrophenol after incubation serve to stop the phosphor-monoesterase activity, to develop yellow colour and to provide quantitative recovery of p-nitrophenol from soils [11]. Phospho-monoesterases activity is expressed as microgram p nitrophenol released g^{-1} soil h^{-1}.

Urease activity

Urease activity in soils involves estimation of urea hydrolysis in soils by determination of urea remaining after incubation of soil with urea solution at 37 0C. The difference between the amount of urea added and that recovered after incubation for a specific time is taken as an estimate of urease activity [12].

Statistical Analysis

To derive valid conclusion data where statistically analysed using Factorial Complete Randomized Design (FCRD) with the appropriate ANOVA. Overall significance of treatments was tested by F-test and further comparisons were made with the critical difference (CD) 1% degree of significance to draw the valid differences among the treatments [13].

Result and Discussion

Dehydrogenase activity

Data pertaining to dehydrogenase activity of maize rhizosphere at various growth periods significantly influenced by different bacterial K-isolates and levels of K fertilization [Table-1]. Comparing the mean data at 30 DAS, JHG11 showed the highest dehydrogenase activity which was 15.98% greater than the control. APN7 and RJJ4 showed at par values of dehydrogenase activity. Increasing the levels of K fertilization increased the DHA activity. It might be due to enhanced growth of the crops. Many workers reported that Microbial activities increase the DHA in the rhizosphere and enhance availability of food materials for its growth. Variation in DHA activity in the rhizosphere due to plant genetic as well microbial species associated with it ([14, 15].

Dehydrogenase activities in rhizosphere soils of maize were significantly higher in treatment receiving bacterial K-isolates and mineral K at all the crop stages when compared with absolute pots. JHK2 showed the maximum activity when K was applied @ 60 kg K_2O ha^{-1} and it was at par with the activity of BRG 6. The activity of JHK2 was greater than unfertilized pots. It was observed a marked increase in dehydrogenase activity in the soil of organic farms than that of conventional farms using K-solubilizers in the major cropping system *viz*. cotton, sugarcane, maize, jowar and vine yard [16]. With 0 and 60 kg K_2O ha^{-1}, JHK2 caused 20.15% and 15.19% greater dehydrogenase activity than control, respectively. At harvest, DHA activities decreased with the addition of fertilizer. BRG6 with 60 kg K_2O ha^{-1} of K resulted highest activity of dehydrogenase which was 5.52% greater than control. Increase in dehydrogenase activity due to application of K-solubilizing bacteria [17]. They also stated that the enzymatic activities are often used as indices of microbial growth, which further may reflect the microbial respiration and the potential capacity of soil to perform biological transformation of several nutrients. Soil dehydrogenase activity is only present in viable cells and it is thought to reflect the total range of oxidative activity of soil microflora and consequently may be an important indicator of microbial activity [18].

Urease activity

JHK2 showed the highest mean activity at 30 DAS (0.457mg NH4 g^{-1} soil $hour^{-1}$) being 15.04% higher than un-inoculated control [Fig-1] APN7 gave minimum urease activity and it was 6.06 % higher than un-inoculated control. It was evident from the data that with increase in the levels of mineral K fertilization urease activity increased. JHK2 had the highest activity at 60 kg K_2O ha^{-1} of K fertilization. The experimental treatments had a significant impact on rhizosphere soil enzyme activity. When K was applied @ 60 kg K_2O ha^{-1}, the urease activity of JHK2 and JHG11 was at par to each other. Application of 40 kg K_2O ha^{-1} of K, APN7 and RJJ4 resulted similar activities. At harvest, APN7 showed the highest mean activity. 60 kg K_2O ha^{-1}, RJJ 4 showed the highest mean activity which was 5.03% greater than control. Application 20 kg K_2O ha^{-1} and 40 kg K_2O ha^{-1} did not varied much but at 60 kg K_2O ha^{-1} higher value was recorded. Similar results were also found by Dotaniya [19].

Maximum root exudates secretion might have led to higher microbial activity in rhizosphere. Highest enzyme activity was found at the dough stage of the wheat crop followed by the jointing and maturity stages. Rhizosphere microorganisms gather in rhizosphere soil and use rhizosphere exudates as their major nutrients [17].

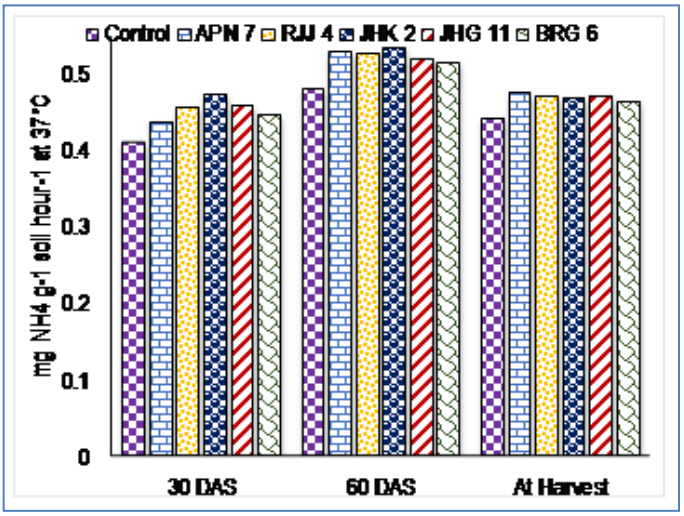

Fig-1 Effect of K -solubilizers and K levels on Urease (mg NH_4 per gram soil per hour at 37°C) activity in Rhizospheric soil of maize at different growth periods

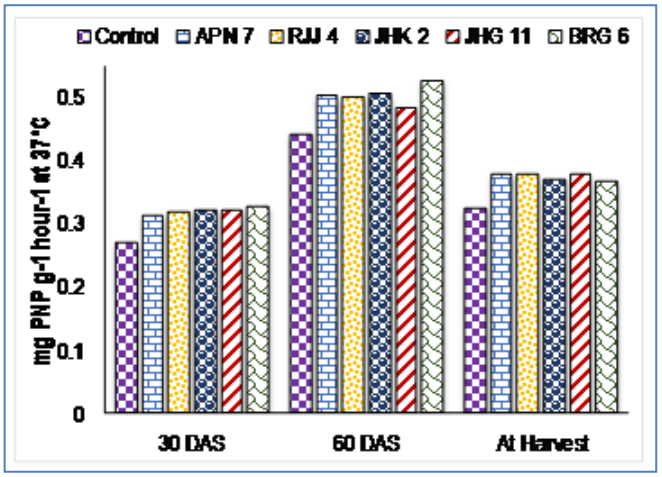

Fig-2 Effect of K -solubilizers and K levels on phosphatase (mg PNP g-1 hour-1 at 37°C) activity in rhizospheric soil of maize at different growth periods

Alkaline phosphatase activity

Influence of different bacterial isolates and levels of K fertilization on alkaline phosphatase activity in rhizosphere of maize at various growth periods have been presented in [Fig-2]. At 30 DAS, BRG 6 showed the highest mean alkaline phosphatase activity which was 20.22% higher than control. Application of K fertilizer increased the alkaline phosphatase activity. BRG 6 had also the highest activity during 40 kg K_2O ha^{-1} of K application. 60 kg K_2O ha^{-1} resulted in the highest alkaline phosphatase activity of APN 7 which was at par with JHG 11. The mean activity of BRG 6 was also pronounced at 60 DAS and maintained its status when K was applied @ 60 kg K_2O ha^{-1}. Similar observations about the effect of different micro-organism on alkaline phosphatase activity also have been reported in maize [20, 21]. Alkaline phosphatase activity influenced by

crop growth stages. It might be due to secretion of root exudates and biochemical changes in plant system [22].

At harvest, RJJ 4 caused highest mean alkaline phosphatase activity which was 17.28% higher than control. Increasing the level from 20 to 60 kg K_2O ha^{-1}, RJJ 4 retained its maximum activity. When K was applied @ 40 kg K2O ha^{-1}, RJJ 4 and JHG 11 showed similar activity and 14.63% higher activity than control. In the entire crop period, the alkaline phosphatase activity increased initially at 30 DAS and then declined with the age of the crop. These observations are in accordance with the findings of Singaram and Kamalakumari [23] where they also noticed similar trend in alkaline phosphatase activity in maize rhizosphere. Dotaniya reported [24] higher amount of root exudation in initial stage of rice growth which enhances density and activity of microorganisms in crop rhizosphere and modifies nutrient concentrations in soil solution. Further, Dotaniya [19] also stated that alkaline phosphatase activity of soil increased up to 75 DAS of maize and declined thereafter.

Table-1 *Effect of K -solubilizers and K levels on dehydrogenase (µg TPF g^{-1} day^{-1}) activity in Rhizospheric soil of maize at different growth periods*

Treatment	30 DAS					60 DAS					At Harvest				
	Levels of potassium (K kg ha^{-1})					Levels of potassium (K kg ha^{-1})					Levels of potassium (K kg ha^{-1})				
	0	20	40	60	Mean	0	20	40	60	Mean	0	20	40	60	Mean
Control	25.3	26.6	27.6	28.3	26.9	27.2	30.8	32.4	32.6	30.7	26.0	28.6	31.2	32.6	29.6
APN 7	29.9	26.8	31.2	31.7	29.9	29.0	31.4	34.2	36.4	32.7	27.3	29.7	32.0	33.5	30.6
RJJ 4	28.2	30.5	28.9	30.7	29.6	31.1	31.8	35.3	36.8	33.8	27.5	30.1	31.7	33.2	30.6
JHK 2	30.4	30.7	31.0	32.6	31.2	29.5	32.2	36.2	37.4	33.8	27.7	32.4	32.1	34.0	31.6
JHG 11	26.8	30.1	29.1	30.0	29.0	31.4	32.1	34.8	36.2	33.6	28.2	31.9	32.4	33.6	31.5
BRG 6	28.9	27.7	31.6	32.2	30.1	30.9	33.1	35.8	36.2	34.0	28.9	31.0	31.5	34.4	31.5
Mean	28.2	28.7	29.9	30.9	-	29.8	31.9	34.8	35.9	-	27.6	30.6	31.8	33.6	-

	30 DAS			60 DAS			At harvest		
	Isolates (I)	Levels(L)	I × L	Isolates (I)	Levels (L)	I × L	Isolates (I)	levels (L)	I × L
SEm ±	0.18	0.12	-	0.12	0.08	-	0.13	0.09	-
CD (P=0.01)	0.68	0.45	NS	0.47	0.31	NS	0.50	0.34	NS

Conclusion

It was concluded that the maize play a vital role in Indian food grain production but the cost of production is increasing due to fertilizers especially potassium. It increases the cost of cultivation and reduces the profit of farmers. Experimental data showed that maximum urease, dehydrogenase and alkaline phosphatase activities were recorded at 60 DAS of maize growth in comparison to 30 DAS and harvest. Application of JHK2 significantly caused highest enzymatic activity at all the growth periods. These enzyme activities associated with higher availability and uptake of nutrients.

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