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Effect of Dual Inoculation of Potassium Solubilizing Bacteria and Phosphorus Solubilizing Bacteria on Microbiological Changes, Enzyme Activity in Rhizosphere of Maize

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A field was carried out at University of Agricultural Sciences Dharwad to evaluate the Effect of dual inoculation of potassium solubilizing bacteria (KSB) and phosphorus solubilizing bacteria (PSB) on microbiological changes, enzyme activity in rhizosphere of maize crop. Bacterial isolates of potassium solubilizing bacteria (KSB) and phosphorus solubilizing bacteria (PSB) were collected from Department of Agriculture Microbiology, University of Agricultural Sciences, Dharwad. Two strains of present study *viz.,* KSB 11 and KSB 27 in combination with PSB 98(2) showed better performance under field condition. Microbial population (KSB and PSB) and enzyme activities (Dehydrogenase, Urease and Phosphatase) in rhizosphere soil of maize were significantly increased in the treatment inoculated with KSB + PSB as compared to control and single inoculation. The maximum microbial population and enzyme activities were recorded in the treatment KSB 11 + PSB 98 (2) + RDN (P and K at 75%).

Key words: Potassium solubilizing bacteria (KSB), Phosphorus solubilizing bacteria (PSB), K –Potassium, P – Phosphorus, Population, Dehydrogenase, Urease and Phosphatase.

Maize (*Zea mays* L.) belongs to family Poaceae and highly cross pollinated crop. Origin of maize is from Central America and Mexico. Bihar, Uttar Pradesh, Madhya Pradesh, Rajasthan, Andhra Pradesh and Karnataka were major maize producing states in Indian. Maize is called as 'queen of cereals' because of its higher grain yield under different climatic conditions. Potassium, phosphorus and nitrogen were essential mineral elements which are necessary for good plant growth and reproduction for more yield. Nitrogen, Phosphorus and Potassium are mobile nutrients which were present in insoluble form but by microbial process helps plants to easily absorb these essential elements.

Phosphorus is a second most important and potassium is the third most important macronutrients for crop plants. Which are directly involved in the energy transfer and protein metabolism in crop plants. During early growth stage of the plant supply of phosphorus is important in initialization of flower for its reproductive parts (Tisdale and Nelson, 1968). Necrosis, interveinal chlorosis and reduction in the rate of photosynthesis is caused by deficiency of potassium. Phosphorus deficiency leads to purple pigment of leaves, stunted plant growth and delay in plant development.

Phosphorus and Potassium are the essential macronutrients for plant growth and development. These two essential nutrients are commonly added as fertilizer to increase yield in

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crop plants. Phosphorus solubilizing bacteria (PSB) have been used to convert insoluble rock phosphate material into soluble form which is available for plant growth. Conversion of unavailable form of Phosphorus and Potassium is by different processes like acidification, chelation, exchange reactions and strong organic acids production, which was becomed indicators for isolation of phosphorus solubilizing bacteria (PSB). Potassium solubilizing bacteria (KSB) are able to solubilize primary K minerals, such as mica, illite and orthoclases, through production organic acids. A combination of application of rock P and K materials with co-inoculation of Phosphorus solubilizing bacteria and Potassium solubilizing bacteria provide a faster and continuous supply of P and K for optimal plant growth (Han and Lee, 2005).

The use of plant growth promoting rhizobacter (PGPR) like phosphate and potassium solubilizing bacteria (PSB and KSB) as biofertilizers, was suggested as a sustainable solution to improve essential nutrient availability for crop plants to increase the production (Badar *et al.*, 2006).

MATERIAL AND METHODS

The bacterial isolates of potassium solubilizing bacteria (KSB -11 and KSB -27) and phosphorus solubilizing bacteria [PSB 98 (2)] were collected from Department of Agriculture Microbiology, College of agriculture, University of Agricultural Sciences, Dharwad.

The field experiment was conducted to study the "Effect of dual inoculation of potassium solubilizing bacteria and phosphorus solubilizing bacteria on microbiological changes, enzyme activity in rhizosphere of maize under field condition" over the varied levels of K and P fertilizers control at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. The experiment was laid out in Randomized Complete Block Design (RCBD) with twelve treatments and three replication. N was applied as per recommended dose to Maize crop.

The KSB – 11, KSB -27 and PSB - 98 (2) for the treatments were grown on (Aleksandrov media for KSB isolates and Pikovskyas media for PSB isolate) medium for 48 hr. After this all the isolates were mixed with the sterilized lignite powder separately then the seed treatment were done according to treatment details selected for field experiment. After inoculation of seeds with KSB and PSB isolate line sowing was done in field.

Treatments includes were T_1 - Potassium and phosphorus control 1+ RDN (P and K at 75%), T_2 - Isolate KSB 11 + RDN (P and K at 75%), T_3- Isolate KSB 27 + RDN (P and K at 75%), T_4- Isolate PSB 98 (2) + RDN (P and K at 75%), T_5- Isolate KSB 11 + PSB 98 (2) + RDN (P and K at 75%), T_6 - Isolate KSB 27 + PSB 98 (2) + RDN (P and K at 75%), T_7- Potassium and phosphorus control 2 + 100% RDF, T_8-Isolate KSB 11 + 100% RDF, T_9- Isolate KSB 27 + 100% RDF, T_{10}- Isolate PSB 98 (2) + 100% RDF, T_{11}- Isolate KSB 11 + PSB 98 (2) + 100% RDF, T_{12}- Isolate KSB 27 + PSB 98 (2) + 100% RDF.

RESULTS AND DISCUSSION

The significantly higher rhizosphere microbial population were recorded at 30, 60 DAS and at harvest in the treatment receiving dual inoculation of PSB and KSB. Dual inoculation with PSB and KSB inoculated plants were recorded higher microbial population as compared with single treatment and control plants. At 60 DAS there were increase in microbial population and decrease in microbial population was observed at harvest.

In the table 1 indicated that the highest PSB population was recorded in the treatment KSB 11 + PSB 98 (2) + RDN (P and K at 75%) (30.05 cfu x 10^4/g soil) at 60 DAS and lowest PSB population was recorded in the treatment Potassium and Phosphorus control 1 + RDN (P and K at 75%) (9.87 cfu x 10^4/g soil) at 30 DAS.

The maximum KSB population was recorded in the treatment KSB 11 + PSB 98 (2) + RDN (P and K at 75%) (22.54 cfu x 10^4/g soil) at 60 DAS and the treatment Potassium and Phosphorus control 1 + RDN (P and K at 75%) (5.08 cfu x 10^4/g soil) at 30 DAS was recorded the least KSB population.

There was significantly increased in the population of PSB and KSB was observed because of dual inoculation of potassium solubilizing bacteria (KSB) and phosphorous solubilizing bacteria (PSB). This may be due to the production growth hormone and release of organic acids. These results were in accordance with the findings of Archana and Savalgi (2007) who reported that the inoculation of potassium solubilizing bacteria (KSB) to the maize crop increased KSB microbial population in rhizosphere maize crop under glasshouse condition.

Similarly, Kunoto and Savalagi (2010) recorded the significantly increased the population of PSB and KSB in rhizosphere of maize crop under glasshouse condition by dual inoculation of potassium solubilizing bacteria (KSB) and phosphorous solubilizing bacteria (PSB).

The results were also comparable with the findings of Kim *et al.*, (2003). Increase in microbial population is may be due to the higher available P and K by the solubilization of P and K by inoculated PSB and KSB isolates [Hu *et al.*, (2006) and Lin *et al.*, (2002)].

Table 1. Influence of dual inoculation of potassium solubilizing bacteria (KSB) and phosphorus solubilizing bacteria (PSB) on PSB population (cfu x 10^4/g soil) in maize rhizosphere

S. No.	Treatments	30 DAS	60 DAS	90 DAS
T_1	Potassium and Phosphorus control 1+ RDN (P and K at 75%)	9.87	16.29	14.11
T_2	KSB 11 + RDN (P and K at 75%)	15.06	24.93	19.73
T_3	KSB 27 + RDN (P and K at 75%)	14.43	23.47	18.83
T_4	PSB 98 (2) + RDN (P and K at 75%)	15.78	25.06	20.28
T_5	KSB 11 + PSB 98 (2) + RDN (P and K at 75%)	20.54	30.05	23.71
T_6	KSB 27 + PSB 98 (2) + RDN (P and K at 75%)	20.74	28.58	25.09
T_7	Potassium and Phosphorus control 2 + 100% RDF	11.55	16.97	14.84
T_8	KSB 11 + 100% RDF	13.25	24.90	17.44
T_9	KSB 27 + 100% RDF	14.84	23.43	18.41
T_{10}	PSB 98 (2) + 100% RDF	16.11	26.07	21.02
T_{11}	KSB 11+PSB 98(2) + 100% RDF	21.07	29.01	24.77
T_{12}	KSB 27 + PSB 98(2) + 100% RDF	19.24	28.70	24.48
	S. Em ±	1.02	1.74	1.35
	CD @ 5%	3.00	5.10	3.97

Recommended N was applied to all the treatments.
RDN- Recommended dose of nitrogen, KSB- Potassium solubilizing bacteria, RDF- Recommended dose of fertilizer.

Table 2. Influence of dual inoculation of potassium solubilizing bacteria (KSB) and phosphorus solubilizing bacteria (PSB) on KSB population (cfu x 10^4/g soil) in maize rhizosphere

S. No.	Treatments	30 DAS	60 DAS	90 DAS
T_1	Potassium and Phosphorus control 1 + RDN (P and K at 75%)	5.08	10.44	7.43
T_2	KSB 11 + RDN (P and K at 75%)	7.63	17.97	12.03
T_3	KSB 27 + RDN (P and K at 75%)	8.05	18.10	13.37
T_4	PSB 98 (2) + RDN (P and K at 75%)	8.17	17.91	13.29
T_5	KSB 11 + PSB 98 (2) + RDN (P and K at 75%)	12.29	22.54	17.11
T_6	KSB 27 + PSB 98 (2) + RDN (P and K at 75%)	11.45	21.40	16.90
T_7	Potassium and Phosphorus control 2 + 100% RDF	5.12	12.05	7.45
T_8	KSB 11 + 100% RDF	9.16	18.53	15.03
T_9	KSB 27 + 100% RDF	8.83	18.21	15.85
T_{10}	PSB 98 (2) + 100% RDF	10.44	19.51	14.25
T_{11}	KSB 11+PSB 98 (2) + 100% RDF	11.13	21.43	15.79
T_{12}	KSB 27 + PSB 98 (2) + 100% RDF	11.25	20.36	16.67
	S. Em ±	0.61	1.16	0.93
	CD @ 5%	1.79	3.42	2.73

Recommended N was applied to all the treatments.
RDN- Recommended dose of nitrogen, KSB- Potassium solubilizing bacteria RDF- Recommended dose of fertilizer.

At 30, 60 DAS and at harvesting the Dehydrogenase, urease and phosphatase were significantly highest with in the treatments receiving both PSB and KSB bacterial isolates. Higher Dehydrogenase, urease and phosphatase activity were in recorded in all the treatments which were inoculated with PSB and bacterial isolates as compared to single treatment and control plot. There were increase in enzymes activity at 60 DAS but the decrease was observed at harvest stage.

The data pertaining to Dehydrogenase, Urease and Phosphatase activity were given in tables 3, 4 and 5 respectively. The highest Dehydrogenase, urease and phosphatase activity was recorded in the treatment KSB 11 + PSB 98 (2) + RDN (P and K at 75%) (5.65 µg TPF/g soil/day, 37.46 µg /NH_4^+ -N/g soil/day and 28.52 µg PNP/g soil/hr respectively) at 60 DAS. The treatment Potassium and Phosphorus control 1 + RDN (P and K at 75%) (1.43 µg TPF/g soil/day, 9.50 µg /

Table 3. Effect of dual inoculation of potassium solubilizing bacteria (KSB) and phosphorus solubilizing bacteria (PSB) on Dehydrogenase activity (µg TPF/g soil/day)

S. No.	Treatments	30 DAS	60 DAS	90 DAS
T_1	Potassium and Phosphorus control 1+ RDN (P and K at 75%)	1.43	2.61	1.85
T_2	KSB 11 + RDN (P and K at 75%)	1.65	3.75	2.44
T_3	KSB 27 + RDN (P and K at 75%)	2.13	3.71	2.48
T_4	PSB 98 (2) + RDN (P and K at 75%)	1.77	4.00	3.13
T_5	KSB 11 + PSB 98 (2) + RDN (P and K at 75%)	3.82	5.65	4.83
T_6	KSB 27 + PSB 98 (2) + RDN (P at K at 75%)	3.08	4.43	3.37
T_7	Potassium and Phosphorus control 2 + 100% RDF	1.67	2.62	2.88
T_8	KSB 11 + 100% RDF	2.55	4.38	3.46
T_9	KSB 27 + 100% RDF	2.50	5.02	3.73
T_{10}	PSB 98 (2) + 100% RDF	2.87	4.12	3.54
T_{11}	KSB 11+PSB 98(2) + 100% RDF	3.56	5.33	4.36
T_{12}	KSB 27 + PSB 98(2) + 100% RDF	3.66	5.58	4.41
	S.Em±	0.16	0.22	0.18
	CD @ 5%	0.47	0.66	0.54

Recommended N was applied to all the treatments.
RDN- Recommended dose of nitrogen, KSB- Potassium solubilizing bacteria, RDF- Recommended dose of fertilizer.

Table 4. Effect of dual inoculation of potassium solubilizing bacteria (KSB) and phosphorus solubilizing bacteria (PSB) on the phosphatase activity (µg PNP/g soil/hr)

S. No.	Treatments	30 DAS	60 DAS	90 DAS
T_1	Potassium and Phosphorus control 1+ RDN (P and K at 75%)	12.40	16.28	13.40
T_2	KSB 11 + RDN (P and K at 75%)	14.72	20.23	16.10
T_3	KSB 27 + RDN (P and K at 75%)	17.30	19.93	20.10
T_4	PSB 98 (2) + RDN (P and K at 75%)	17.26	21.32	20.20
T_5	KSB 11 + PSB 98 (2) + RDN (P and K at75%)	17.77	28.52	22.10
T_6	KSB 27 + PSB 98 (2) + RDN (P and K at 75%)	20.35	24.87	24.60
T_7	Potassium and Phosphorus control 2+ 100% RDF	14.85	16.60	15.90
T_8	KSB 11 + 100% RDF	16.54	21.08	19.10
T_9	KSB 27 + 100% RDF	15.01	19.99	16.00
T_{10}	PSB 98 (2) + 100% RDF	17.36	23.15	21.10
T_{11}	KSB 11+PSB 98(2) + 100% RDF	17.83	24.83	21.60
T_{12}	KSB 27 + PSB 98(2) + 100% RDF	18.61	26.26	23.00
	S. Em ±	0.99	1.45	1.14
	CD @ 5%	2.92	4.27	3.34

Recommended N was applied to all the treatments.
RDN- Recommended dose of nitrogen, KSB- Potassium solubilizing bacteria, RDF- Recommended dose of fertilizer.

NH_4^+ -N/g soil/day and 12.40 µg PNP/g soil/hr) was recorded lowest enzymes activity at 30 DAS.

In the present investigation the Enzyme activity (Dehydrogenase, Phosphatase and Urease activity) in plant rhizosphere significantly highest with the application of KSB + PSB as compared to the control.

In the entire crop period, the enzymes activity increased initially at 60 DAS and then declined with the age of the crop. Thease results were in comparable with the findings of Kunoto and Savalagi (2010) who were reported that increasing in Dehydrogenase, Urease and Phosphatase activities in rhizosphere of maize crop under glassnhouse condition by the inoculation of KSB 11, KSB 16 and KSB 27 in combination with PSB 98(2).

These observations were also in accordance with the findings of Singaram and Kamalakumari (1995) they recorded the similar trend in phosphatase, dehydrogenase and urease activity in maize rhizosphere. The variation in the urease, dehydrogenase and phosphatase were influenced by different inoculation treatments. More microbial population were increases the enzyme activities which were regulated by the soil characters like organic carbon, pH and nutrient status (Nagaraja *et al.*, 1998).

Table 5. Effect of dual inoculation of potassium solubilizing bacteria (KSB) and phosphorus solubilizing bacteria (PSB) on the urease activity (µg /NH_4^+ -N/g soil/day)

S. No.	Treatments	30 DAS	60 DAS	90 DAS
T_1	Potassium and Phosphorus control 1+ RDN (P and K at 75%)	9.50	13.23	10.09
T_2	KSB 11 + RDN (P and K at 75%)	14.86	16.69	16.79
T_3	KSB 27 + RDN (P and K at 75%)	15.55	17.84	17.56
T_4	PSB 98 (2) + RDN (P and K at 75%)	14.47	18.66	20.81
T_5	KSB 11 + PSB 98 (2) + RDN (P and K at 75%)	29.70	37.46	34.16
T_6	KSB 27 + PSB 98 (2) + RDN (P and K at 75%)	27.02	33.19	28.35
T_7	Potassium and Phosphorus control 2 + 100% RDF	9.73	12.32	11.32
T_8	KSB 11 + 100% RDF	22.03	24.41	21.48
T_9	KSB 27 + 100% RDF	17.34	21.55	18.29
T_{10}	PSB 98 (2) + 100% RDF	16.45	21.08	18.59
T_{11}	KSB 11+PSB 98(2) + 100% RDF	26.56	31.61	30.07
T_{12}	KSB 27 + PSB 98(2) + 100% RDF	27.80	34.57	32.19
	S. Em ±	1.05	1.57	1.77
	CD @ 5%	3.08	4.61	5.21

Recommended N was applied to all the treatments.
RDN- Recommended dose of nitrogen, KSB - Potassium solubilizing bacteria, RDF- Recommended dose of fertilizer.

CONCLUSION

Significantly increase in microbial (PSB and KSB) population and enzymes activity (Dehydrogenase, Urease and Phosphatase) in maize rhizosphere were seen in the treatment inoculated with both PSB and KSB bacterial isolates as compare to control plot. Dual inoculation of PSB and KSB to the maize crop have better performance under field condition.

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