Biological Forum – An International Journal 15(7): 66-70(2023)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Influence of Different Potassium Solubilizing Microbial Inoculants on Enzyme Activities and Biological Properties of Soil

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(Received: 17 April 2023; Revised: 29 April 2023; Accepted: 21 May 2023; Published: 05 July 2023)
(Published by Research Trend)

ABSTRACT: Potassium (K) is an important nutrient required for plant growth. K present in soil include both available and non-available form and among this non-availble form is comparatively higher in concentration. Thus, many are following the addition of chemical fertilizers, and that finally depletes the soil quality. Here comes the importance of potassium solubilizing bacteria (KSB) because which could solubilize the insoluble K and make it available for plant uptake. To study this a pot trail was carried with soil planted brinjal to assess the influences of potassium solubilizing bacteria (KSB) on the enzyme activities and microbial count in soil.

The effects were examined of nine inoculation-treatments (KSB-W1, KSB-PD-3-A, KSB-NP-3, KSB-PD-1-A, KSB-M-1, KSB-PD, KSB-M-2, KSB-PD-1-B, KSB-M-3) and a non-inoculation (control) treatment on the enzyme-activities and the microbial-count in brinjal soil. The results showed that the Use of *Pseudomonas* sp (KSB-PD-1-A) inoculation significantly improved dehydrogenase activity in soil. Whereas highest increase in activity of acid and alkaline phosphates in soil was found at KSB strain *Bacillus* sp (KSB-PD-3-A). The soil microbial- population at all growth stages of brinjal crop was also increased with *Pseudomonas* sp (KSB-PD-1-A) along with recommended dose of fertilizers.

Keywords: KSB, soil biological properties, enzyme activity, microbial count.

INTRODUCTION

In order to full fill the need of the growing population, farming must be concentrated and sustainable in the future. Nevertheless, it is well identified that the food invention by the agriculture sector cannot be normally continued unless the nutrients removed from soil because of enhanced crop production are changed. Required quantity of nutrients are lacking in agricultural field, thus leads to reduced crop production. To escape from this crisis growers have started to use chemical fertilizers (Glick, 2012). However, the chemicals improved the crop production, they reduce the soil quality. It is understood that the continues incorporation of chemical fertilizers have negative impacts on the environment (Adesemoye and Kloepper 2009).

Potassium (K), a major nutrient essential for growth of plant, performs an principal part in concluding the production rate and metabolism of crops (Salvo *et al.*, 2017). Still, just only <10% of the total content of K present could be taken by plants from the soil. Mander *et al.* (2012) reported that K present in the form of feldspar and mice is hard to get solubilized in soil so its not easily available for plant uptake. Now a days chemical fertilizers are taking key role in crop improvement so many are following chemical fertilizers (Khan *et al.*, 2019). But, this leads to many environmental pollutions due to the less importance given to sustainable soil practices under chemical fertilization (Huang *et al.*, 2021).

From many studies we could conclude that micro level soil community is capable to impact soil fertility by different soil processes *viz.*, mineralization, decomposition, and storing/distribute nutrients (Parmar and Sindhu 2013). Microbes like different strains of bacteria and fungus have proven for their ability to solubilize insoluble form of K by number of mechanisms like making of organic and inorganic acids, polysaccharides, acidolysis, complexolysis, polysaccharides, chelation, and ion exchange reactions. Between these micro-organisms, K solubilizing bacteria (KSB) have got the consideration of agriculturists as soil inoculum to encourage the plant growth and production.

Ding *et al.* (2021) reported that there are some bacteria (KSB) they could solubilize the insoluble source of K and make it available for plant uptake. Zhao *et al.* (2023) also stated that use of bacterial inoculants could improve plant growth parameters plant metabolism.

The KSB are applicable in liberating K from inorganic and unsolvable pools of total soil K by solubilization and also it could enhance the microbial count and thereby enzyme activity in soil (Archana *et al.*, 2013). Patil *et al.* (2022) found out that treating ground nut seeds with potassium solubilizing microbes bring about in better growth, protein content, pod yield compared to package of practices. Hence, the current study was taken to know the influence of different potassium solubilizing microbial inoculants on enzyme activities and biological properties of soil.

MATERIALS AND METHODES

A pot trial was done with brinjal grown soil to assess the influences of potassium solubilizing bacteria (KSB) on the soil enzyme activities and microbial count. The effects were examined of 9 inoculation treatments (KSB-W1, KSB-PD-3-A, KSB-NP-3, KSB-PD-1-A, KSB-M-1, KSB-M-2, KSB-PD, KSB-PD-1-B, KSB-M-3) and a non-inoculation treatment (control) on the activities of enzyme and the microbial count in brinjal soil. Soil samples were gathered from every single pot at fruit development and harvesting-stage. The samples were systematically mixed up and carried to the lab for further analysis.

A. Dehydrogenase-enzyme activity

It was find out by TTC technique as described by Klein *et al.* (1971). One grams of soil was incubated for 24 h at 28+0.5 ºC in 0.2 ml of a 3% TTC solution (3 g TTC in 10 ml distilled water) and 0.5 ml of 1% glucose solution. Two droplets of conc. H_2SO_4 were also added just after the incubation to stop the reaction. The samples treated was then mixed with 10 ml of methanol and shaken for 30 min at 250 rpm. Allow to wait for 6h. The optical density of the color (red) was identified at 485 nm using UV; Vis spectrophotometer. dehydrogenase activity was presented as µg TPF g^{-1} of soil $24h^{-1}$. Acid and Alkaline Phosphomonoesterases activity (soil). Acid and Alkaline phosphatase enzymatic activity was calculated with spectrophotometry as defined by Tabatabai and Bremnar (1969). soil of 1g was taken in a 50 ml flask and added toluene (0.25 ml) and MUB buffer (4 ml) and solution of p-nitrophenolphosphate made with same buffer. Then the sample incubate after thorough mixing for 1 h (37ºC), to this flask $CaCl_2$ (1 ml 0.5 M) and NaOH (4 ml 0.5 M) were added after 1hr of incubation. The soil suspension (colored) was filtered over Whatman filter paper and the absorbance was noticed at 400 nm. The activity of phosphatase was noted as µg *p*-Nitrophenol- g^{-1} of soil h^{-1}.

B. Soil microbial count

For screening of different bacteria, fungi and actinomycetes from experimental soil 3 various media were used for precise group of micro flora. Soil microbial counts were estimated using serial dilution method as described by Parmer and Schmidt (1964).

RESULTS AND DISCUSSION

A. Effect of diverse potassium solubilizing microbial inoculants on activity of enzyme in soil

The bio-chemical nature of soil have often been projected as early and sensitive needles of soil ecosystem health. Soil enzymes activities specify the energy level of all the different bio-chemical reactions in soil and perform as main indicator of biological properties of soil. Soil enzymes show an vital part in energy transfer, organic matter decomposition environmental value, crop productivity and nutrient cycling. calculation of enzymatic activity in combination with count of number of key micro-organisms provides sensitive information of the changes occurring in soil. The data regarding enzyme activity in soil are narrated in Table 1.

Dehydrogenase activity in soil. The examination of the data given in Table 1 on the effect of inoculation on soil dehydrogenase activity revealed that there was significant variation between potassium solubilizing microbial strains and uninoculated control. It was found to be decreased as growth period extends from 90 to 150 days (31.26 to 29.70 µg TPF g^{-11} soil 24 hr^{-1}). The KSB strain *Pseudomonas* sp (KSB-PD-1-A) (41.09 and 39.23 µg TPF g^{-1} soil 24 hr^{-1}) and *Pseudomonas* sp (KSB-M-1) (40.17 and 38.91 µg TPF g^{-1} soil 24 hr^{-1}) were found significantly better as compared to other KS strains and uninoculated control in increasing dehydrogenase of soil. Whereas, lowest dehydrogenase activity was found in uninoculated control (22.73 and 21.49 µg TPF g^{-1} soil 24 hr^{-1}).

Our results agree with those already reported Basavesha (2013) and Chishi (2010), they found significantly higher dehydrogenase activity at inoculated treatments than control. Dehydrogenase enzymes provide an signal of microbial population in soil. More activity means the inoculated fungal strains were able to take possession of the rhizosphere soil having pomegranate (Maity *et al*., 2014). Dehydrogenase activity have ability to give a unique value of over-all activity of soil microorganisms and integrative-biological-assessment relating to biological activity or bio-chemical processes of soils due to its association to soil biology. Dehydrogenase activity replicates the oxidative activity of metabolism of soil microflora and can be used as an pointer of microbial - activity in soils (Beura and Rakshit 2013).

Alkaline phosphatase activity in soils. Effect of potassium solubilising microbial inoculants on periodical changes in alkaline phosphatase activity in soil was analysed during experimental period and data is presented in Table 1. Alkaline phosphatase activity decreased from flowering to harvesting stage of crop. (122.64 to 121.85 µg PNP- g^{-1} of soil). Significantly highest alkaline phosphatase activity was noted in treatment *Bacillus* sp (KSB-PD-3-A) (134.12 and 132.54 µg g^{-1} of soil) and it was on par to *Bacillus* sp (KSB-W1) (133.35 and 132.077 µg g^{-1} of soil), *Pseudomonas* sp (KSB-M-2) (132.343 and 131.17 µg g^{-1} of soil) and *Pseudomonas* sp (KSB-PD-1-A) (129.79 and 129.06 µg g^{-1} of soil).

However, all the treatments were superior to uninoculated control (98.66 and 98.23 µg g^{-1} of soil) which recorded significantly lowest alkaline phosphatase activity at various sampling intervals, respectively. Ma *et al*. (2022) reported an improvement in enzyme activity in the treatments received biochar along with microbial inoculants.

Table 1: Effect of potassium solubilizing microbial inoculants on enzyme activity in soil.

Treatments	Dehydrogenase (µg TPF g^{-1} soil $24hr^{-1}$)		Alkaline phosphatase (µg p-Nitrophenol g^{-1} soil hr^{-1})		Acid phosphatase (µg p-Nitrophenol g^{-1} soil hr^{-1})	
	90 DAT	150 DAT	90 DAT	150 DAT	90 DAT	150 DAT
T_1: Uninoculated control	22.73	21.49	98.66	98.23	44.50	44.44
T_2: RDF + *Bacillus* sp (KSB-W1)	25.84	23.97	133.35	132.07	63.77	63.41
T_3: RDF + *Bacillus* sp (KSB-PD-3-A)	33.63	32.38	134.12	132.54	68.74	68.30
T_4: RDF + *Bacillus* sp (KSB-NP-3)	26.46	25.22	118.33	117.63	46.67	45.18
T_5: RDF + *Pseudomonas* sp (KSB-PD-1-A)	41.09	39.23	129.79	129.06	67.76	67.49
T_6: RDF + *Pseudomonas* sp (KSB-M-1)	40.17	38.91	103.66	103.35	46.00	45.73
T_7: RDF + *Pseudomonas* sp (KSB-M-2)	36.43	34.55	132.34	131.17	57.07	56.63
T_8: RDF + *Sinorhizobium metallidans* (KSB-PD)	32.68	31.13	125.43	124.97	54.69	54.32
T_9: RDF + *Sinorhizobium metallidans* (KSB-1-B)	28.33	26.46	126.51	125.87	53.88	52.28
T_{10}: RDF + *Sinorhizobium metallidans* (KSB-M-3)	25.22	23.66	124.2	123.56	45.33	44.71
GM	31.26	29.70	122.64	121.85	54.84	54.25
SE$\pm$	1.16	1.03	1.28	1.36	0.71	0.95
CD at 5%	3.52	3.13	4.02	4.09	2.42	2.29
CV %	7.46	6.96	2.09	2.24	2.59	2.48
Initial	23.00		97.54		43.53	

Acid phosphatase activity in soil. Acid phosphatase activity was meaningfully influenced to the inoculation of different potassium solubilising microbial inoculants and is presented in Table 1. Acid -phosphatase-activity in soil was seen significantly highest at flowering stage than that of harvest and ranged from 44.50-68.74 and 44.44-68.30 µg g^{-1} of soil.

Significantly highest acid phosphatase of 68.74 and 68.30 µg g^{-1} of soil was registered at flowering and also at harvest of brinjal crop in the treatment of *Bacillus* sp (KSB-PD-3-A) and it was followed by *Pseudomonas* sp (KSB-PD-1-A) (67.76 and 67.49 µg g^{-1} of soil and *Bacillus* sp (KSB-W1) (63.77 and 63.41 µg g^{-1} of soil), lowest was found in uninoculated control (44.50 and 44.44 µg g^{-1} of soil) at various sampling intervals.

Phosphatase activity decreased as the crop growth period advanced; Our results are corroborate with the results of Beura and Rakshit (2011), they found that alkaline-phosphatase-activity was higher than acid-phosphatase-activity. Phosphatase-activity has been thoroughly correlated with pH. The acid-phosphatases dominate in acid soil and alkaline phosphatase-activity in alkaline soil. Over-all, alkaline-phosphatase is linked with microorganisms while the acid-phosphatase is mostly due to plants. So, rise in microbial biomass might have credited to the practical higher alkaline-phosphatase-activity shadowed by alkaline pH. Our results are also concurrent with the findings of Chishi (2010), the entire crop period the enzyme-activity raised in the starting and then reduced with crop growth. Significantly higher alkaline and acid phosphatase-activities were also observed in inoculated treatments than the control (Maity *et al*., 2014).

B. Effect of different potassium solubilizing microbial inoculants on microbial properties of soil

In order to understand the change in microbial population at different age of the crop the analysis of microbial population was made from different periods like flowering and also at harvest of the crop. Results are summarized in Table 2.

The results clearly indicate that bacterial population in soil gradually decreased from flowering to harvesting stage of crop (129.66 to 127.43 CFU × $10^{-7}g^{-1}$ of soil). Significantly the highest population was found in *Pseudomonas* sp (KSB-PD-1-A) (182.33 and 181 CFU. × 10^{-7} g^{-1} of soil) followed by *Pseudomonas* sp (KSB-M-1) (179 and 176.33 CFU × 10^{-7} g^{-1} of soil), *Pseudomonas* sp (KSB-M-2) (175 and 171 CFU × 10^{-7} g^{-1} of soil) which were found to be superior over other treatments at flowering and also at harvest stage of crop, respectively. Whereas, the smallest population was found in uninoculated control (61.667 and 59.333 CFU × 10^{-7} g^{-1} of soil).

Soil fungal count was also affected by the application of various potassium solubilizing bacterial and fungal strains in treatments. Periodical changes are noticed during sampling time. Fungal count in soil was decreased with rise in growth of crop (8.5 to 7.37 CFU × 10^{-4} g^{-1} of soil). At flowering and harvest stage of crop growth, fungal population was varied from 3.33-14.66 and 2-13 CFU × 10^{-4} g^{-1} of soil. Significantly highest population was found in *Pseudomonas* sp (KSB-PD-1-A) (14.66 and 13 CFU × 10^{-4} g^{-1} of soil) followed by *Pseudomonas* sp (KSB-M-1) (12.33 and 11.66 CFU × 10^{-4} g^{-1} of soil), *Pseudomonas* sp (KSB-M-2) (11.33 and 10 CFU × 10^{-4} g^{-1} of soil) which were found to be superior over other treatments at flowering and harvest stage of crop, respectively. While lowest population was observed in control pot (3.33 and 2 CFU × 10^{-4} g^{-1} of soil) in respective sampling stages.

Table 2. Effect of different potassium solubilizing microbial inoculants on microbial properties of soil.

Treatments	Soil bacteria (CFU × 10^{-7} g^{-1} of soil)		Soil fungi (CFU × 10^{-4} g^{-1} of soil)		Soil actinomycetes (CFU × 10^{-5} g^{-1} of soil)	
	90 DAT	150 DAT	90 DAT	150 DAT	90 DAT	150 DAT
T_1: Uninoculated control	61.67	59.33	3.33	2.00	23.00	20.67
T_2: RDF + *Bacillus* sp (KSB-W1)	73.00	72.67	4.33	3.33	28.33	24.00
T_3: RDF + *Bacillus* sp (KSB-PD-3-A)	170.00	167.33	9.33	8.00	75.33	71.00
T_4: RDF + *Bacillus* sp (KSB-NP-3)	82.67	81.00	7.00	5.67	33.00	28.67
T_5: RDF + *Pseudomonas* sp (KSB-PD-1-A)	182.33	181.00	14.67	13.00	105.33	101.33
T_6: RDF + *Pseudomonas* sp (KSB-M-1)	179.00	176.33	12.33	11.67	97.00	94.67
T_7: RDF + *Pseudomonas* sp (KSB-M-2)	175.00	171.00	11.33	10.00	84.00	80.67
T_8: RDF + *Sinorhizobium metallidans* (KSB-PD)	98.33	96.00	7.67	6.67	52.33	48.33
T_9: RDF + *Sinorhizobium metallidans* (KSB-1-B)	152.33	150.33	8.67	7.33	68.33	62.00
T_{10}: RDF + *Sinorhizobium metallidans* (KSB-M-3)	122.33	119.33	6.33	6.00	40.67	37.33
GM	129.66	127.43	8.50	7.37	60.73	56.87
SE±	2.58	2.64	0.35	0.44	2.18	1.16
CD at 5%	7.81	7.98	1.20	1.36	6.63	3.57
CV %	3.98	4.15	8.32	11.88	7.19	4.09
Initial	58		4		21	

The data regarding periodical changes in actinomycetes population is presented in Table 2. It was found to be decreased from flowering to harvest stage of crop (60.73 to 56.87 CFU × 10^{-5} g^{-1} of soil). The results obtained indicated that the Significantly highest population was found in *Pseudomonas* sp (KSB-PD-1-A) (105.33 and 101.33 CFU × 10^{-5} g^{-1} of soil) followed by *Pseudomonas* sp (KSB-M-1) (97 and 94.66 CFU × 10^{-5} g^{-1} of soil), *Pseudomonas* sp (KSB-M-2) (84 and 80.66 CFU × 10^{-5} g^{-1} of soil) which were found to be superior over other treatments at flowering and harvest stage of crop, respectively. While, the minimum population was noted with uninoculated control (23 and 20.66 CFU × 10^{-5} g^{-1} of soil).

Abd El-Ghany *et al.*, (2010) also stated that treatment of wheat plant for two periods with combination of selected microbes significantly improved population of actinomycetes and fungal. Microbial counts were influenced by the treatments applied, time and stage of plant growth. With respect to stage of wheat plant growth, the counts manage to rise significantly near heading stage then reduced towards harvesting. Also Archana (2007) reported that all the inoculated treatments showed maximum population of KSB over absolute control. Trabalsi (2013) also stated that after the application of microbial inoculants the soil microbial community improved compared to no inoculant treatments.

CONCLUSION

All the inoculated treatments had higher enzyme activity and microbial count compared to control. Use of *Pseudomonas* sp (KSB-PD-1-A) inoculation significantly improved dehydrogenase activity in soil. Whereas highest rise in acid and alkaline-phosphates-activity in soil was found at KSB strain *Bacillus* sp (KSB-PD-3-A). The soil microbial-population at various growth stages of brinjal crop was also improved with *Pseudomonas* sp (KSB-PD-1-A) along with recommended dose of fertilizers.

Acknowledgement. We feel thankful to Dr. Z.A. Baba, Incharge of Microbiology and Biofertilizer Unit, SKUAST, Kashmir for offering the microbial strains for this work.

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How to cite this article: Nihala Jabin P. P. and and Syed Ismail (2023). Influence of Different Potassium Solubilizing Microbial Inoculants on Enzyme Activities and Biological Properties of Soil. *Biological Forum – An International Journal*, *15*(7): 66-70.