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Ekta Kumari
Dept. of Agronomy, Department of Mycology and Plant Pathology, Institute of Agril. Sciences, B.H.U., Varanasi, Uttar, Pradesh, India

Avijit Sen
Dept. of Agronomy, Department of Mycology and Plant Pathology, Institute of Agril. Sciences, B.H.U., Varanasi, Uttar, Pradesh, India

VK Srivastava
Dept. of Agronomy, Department of Mycology and Plant Pathology, Institute of Agril. Sciences, B.H.U., Varanasi, Uttar, Pradesh, India

Ram K Singh
Dept. of Agronomy, Department of Mycology and Plant Pathology, Institute of Agril. Sciences, B.H.U., Varanasi, Uttar, Pradesh, India

Y Singh
Dept. of Agronomy, Department of Mycology and Plant Pathology, Institute of Agril. Sciences, B.H.U., Varanasi, Uttar, Pradesh, India

BR Maurya
Dept. of Soil Science, Department of Mycology and Plant Pathology, Institute of Agril. Sciences, B.H.U., Varanasi, Uttar, Pradesh, India

Vijaya P
Agril. Chemistry, Department of Mycology and Plant Pathology, Institute of Agril. Sciences, B.H.U., Varanasi, Uttar, Pradesh, India

B Sarma
Dept. of Plant Physiology, Department of Mycology and Plant Pathology, Institute of Agril. Sciences, B.H.U., Varanasi, Uttar, Pradesh, India

Correspondence
Ekta Kumari
Dept. of Agronomy, Department of Mycology and Plant Pathology, Institute of Agril. Sciences, B.H.U., Varanasi, Uttar, Pradesh, India

Effect of different potassium solubilising bacteria (KSB) and *Trichoderma* on soil microbial status of baby corn (*Zea mays* L.)

Ekta Kumari, Avijit Sen, VK Srivastava, Ram K Singh, Y Singh, BR Maurya, Vijaya P and B Sarma

Abstract
A field experiment was conducted in 2014-15 and 2015-16 in rabi seasons at agricultural research farm of B.H.U. to study the effect of different strain of potassium solubilising bacterias and fungi in combination with various fertility levels on the microbial status and their corresponding impact on soil health. The trial consisting of *Agrobacterium*, *Flavobacterium*, *Rhizobium* and fungal strain of *Trichoderma* and four fertility levels was laid out in a split plot design with 4 replication by keeping fertility levels in the main plot and strains in the subplot. Significantly higher microbial population was recorded by the use of different microbes. Bacteria (141.12 x 10^{-6}), actinomycetes (53.25 x 10^{-5}) and fungal (45.5 x 10^{-4}) population of cfu g^{-1} of soil were found to be the higher with 100% NP+75%K + *Agrobacterium sp.* and it remained at par with 100%NPK could improve and enrich the fertility status and health of the soil thereby reducing the harmful effect of chemicals.

Keywords: Effect, different, potassium, solubilising, bacteria, (KSB), *Trichoderma*, soil microbial, (*Zea mays* L.)

Introduction
The cultivation of baby corn is becoming popular among the growers in rural areas in recent years due to its diverse utility and high net returns (Verma *et al.*, 2012) [12]. Adopting such crops, farmers were able to get higher economic return in short period as compared to traditional crops. Cost effective production and processing of baby corn may occupy an important place in the area of agri-business. India is emerging as one of the potential baby corn producing countries because of its low cost production technology as compared to other countries. In Varanasi region of Eastern Uttar Pradesh, baby corn is rather a new introduction and efforts are required to standardize and economize its cultivation.

Current soil management strategies are mainly dependent on inorganic chemical-based fertilizers, which cause a serious threat to soil health and environment. The exploitation of beneficial microbial strain as a biofertilizer has become of paramount importance in agriculture sector for their potential role in improving soil health and sustainable crop production. The eco-friendly approaches inspire a wide range of application of useful microscopic organisms leading to improved nutrient uptake (Datta *et al*., 2009) [9].

Biofertilizer are organic products containing living cells of different types of micro-organism, which have the ability to convert nutritionally important elements from unavailable to available form through biological process (Vessey, 2003) [1]. Therefore, the inoculations with KSB and other useful microbial inoculants in the soil mandatorily restore and maintain the effective microbial population that solubilizes the chemically fixed form of potassium and made availability of other macro and micronutrients to enrich the soil fertility status (Pathak *et al*., 2009). The potassium solubilizing bacteria is a rhizospheric bacteria which solubilises the insoluble potassium (K) to soluble forms of K for plant growth and yield. The use of potassium solubilizing bacteria as a biological fertilizer was suggested as a solution to improve plant nutrition (Meena *et al*., 2014b) [2]. These KSB can help in enhancing the availability of nutrients playing an essential role in dynamic soil environment by contributing release of key nutrients from primary minerals and ores. These key macronutrients are central for nutrition of microbial population present in the soil and in turn also effectuate the benefit to plant nutritional status (Meena *et al*., 2014a) [3].

Trichoderma asperellum had the ability to promote plant growth through different mechanisms, such as solubilization of phosphates, potassium and minerals such as Fe, Mn and Mg that have important role in plant growth (Mastouri *et al.*, 2010) [4]. *Trichoderma* increases the rate of germination of maize and seedling growth of maize. A number of mechanisms for plant growth promotion by *Trichoderma* have been proposed (Harman *et al.*, 2004) [5]. *Trichoderma* promotes primary root length and root branching in maize by inducing lateral root growth (Bjorkman, 2004) [6]. Combination of *Trichoderma* inoculum and fertilizers performes better than single application of either fertilizer or inoculum. Wu *et al*. (2005) [7] reported that the application of biofertilizer containing mycorrhizal fungus and three species of bacteria significantly increase the growth of maize.

Materials and Methods

The Agricultural Research Farm is situated at a distance of about 10 km from Varanasi Cant railway station. Flanked by left bank of the river Ganges it is situated at the Southern end of Varanasi city. Geographically the farm lies at 25^0 18'N latitude and 88^0 03'E longitude and at an altitude of 128.93 meters above the mean sea level. The experimental site was homogeneous in fertility with even topography, uniform textural make up having assured irrigation and other required facilities. Proper drainage facility was also available in order to remove the excess water if any, during the experimental period.The maximum temperature usually fluctuates between 23.2˚C to 42.8˚C while minimum temperature varies from 7.3 to 28.2˚C. Occasionally extreme of minimum and maximum temperature variations are also realized. Total rainfall received during 2014-15 and 2015-16 was 1008.9 mm and 1248.3 mm, respectively which was 19.17% higher in 2015-16 than 2014-15. The weekly mean maximum relative humidity during the first experimental year (2014-15) ranged from 32 to 96 per cent with an average of 64 per cent and it ranged from 36 to 96 per cent during the crop cycle of baby corn. The weekly mean maximum relative humidity during the second year (2015-16), ranged from 26 to 94 per cent with an average of 60 per cent.

Experimental details

The experiment was laid out in a split plot design with 4 fertility levels (F_1, F_2, F_3 and F_4) in main plot and 4 strains (three bacterial strains of KSB i.e. S_1, S_2, S_3) and one fungal strain of *Trichoderma i.e.* (S_4) as a seed treatment in sub plot for baby corn. The experimental details were 100% NP+25% K (F_1), 100% NP+ 50% K (F_2), 100% NP+75% K (F_3) and 100% NPK (F_4) in the main plot and sub plot treatments (microbial strains) were *Agrobacterium sp.* (S_1), *Flavobacterium sp.* (S_2), *Rhizobium sp.* (S_3) and *Trichoderma sp.* (S_4). The potassium solubilising bacterial strains and fungal strain were applied as seed treatments. The seed was mixed with the broth culture of KSBs and *Trichoderma* strains alongwith gum acacia for 6-8 hrs before sowing. Treatmentwise, the treated seeds were then used for sowing. 50 mL of each strain (broth culture) with 3 mL of gum acacia was applied for 5 kg seeds.

Microbial population (cfu/g of soil)

Microbiological studies in respect of total bacterial, actinomycetes and fungal count were carried out using standard plate count technique (Wollum, 1982) [8]. The microbial population estimation was done before sowing of baby corn, at 60 DAS and after harvest of baby corn followed by before sowing of moong, 60 DAS and at harvest of moong during both the years of experimentation.

Table 1: Temperature and time of incubation for different nutrient media:

S. No.	Media used	Temperature range (ºC)	Incubation period (days)
1.	Nutrient Agar	30-32	4-6
2.	Rose Bengal	28	2
3.	Ken knight and Munniar's Agar	28	7-10

Calculation of microbial count: average no. of colonies per plate for a given dilution was multiplied by dilution factor to obtain the viable count or cfu per gram soil.

Soil microbial population

The data pertaining to soil microbial population (total bacterial, actinomycetes and fungal) were taken as influenced by levels of fertility and application of microbial strains were summarized in Table 2, 3 and 4. It was found that for the second year of succeeding crops sown, the fertility status of the soil has been improved and the population was exhibited more in 2015-16 than the previous year 2014-15.

Table 2: Effect of microbial strains (KSB and *Trichoderma*) and fertility level on bacterial population (colony forming unit) cfu/g of soil.

Treatments	Bacterial population					
	Before sowing, 60DAS, After harvesting of baby corn					
	10^{-6} cfu/g		10^{-6} cfu/g		10^{-6} cfu/g	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
Fertility levels (RDF)						
F_1 (100%NP+25%K)	92.52	95.61	117.45	126.55	99.51	101.74
F_2 (100%NP+50%K)	95.85	99.14	128.56	132.74	108.12	112.56
F_3 (100%NP+75%K)	98.08	109.42	142.65	146.46	126.42	132.41
F_4 (100% NPK)	101.47	112.74	146.78	151.41	129.51	135.89
SEm±	1.16	1.12	1.50	1.63	1.22	1.26
C.D. (P=0.05)	3.42	3.71	4.61	5.39	3.42	3.66

Microbial strains						
S_1 (*Agrobacterium sp.*)	97.14	100.87	138.47	141.12	118.65	121.54
S_2 (*Flavobacterium sp.*)	89.12	91.29	115.98	118.84	98.15	100.41
S_3 (*Rhizobium sp.*)	93.42	95.56	119.89	123.54	104.77	106.87
S_4 (*Trichoderma sp.*)	93.55	97.39	125.17	130.52	109.85	112.54
SEm±	1.14	1.15	1.47	1.58	1.31	1.20
C.D.(P=0.05)	3.55	3.40	4.46	5.22	3.94	3.57

Bacterial population (cfu g^{-1} of soil)

greater number of colonies of bacteria was observed at 100%NP+75%K (F_3) and 100%NPK (F_4) level which remained statistically at par with each other. The fertility level of F_3 on the basis of average of two year data exhibited 18.49% more bacterial colonies than 100%NP+25%K (F_1) at 60 DAS. No. of bacterial colonies increased upto 60 DAS at 10^{-6} dilutions of cfu/g of soil after which there was a decrease in the number at harvest of baby corn. The response of bacterial population to different fertility levels was in the order of F_4, F_3> F_2> F_1.

Microbial strains played a significant role in maintaining fertility. *Agrobacterium sp*. produced maximum no. of bacterial colonies which was on an average 19.07% more than *Flavobacterium sp*. (S_2) at 60 DAS. Higher no. of colonies was found at 60 DAS of the crop growth after which there was a decrease.

Table 3: Effect of microbial strains (KSB and *Trichoderma*) and fertility level on actinomycetes population (colony forming unit) cfu/g of soil.

Treatments	Actinomycetes population					
	Before sowing, 60DAS, After harvesting of baby corn					
	10^{-5} cfu/g		10^{-5} cfu/g		10^{-5} cfu/g	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
Fertility levels (RDF)						
F_1 (100%NP+25%K)	39.99	41.87	44.24	46.49	41.23	43.85
F_2 (100%NP+50%K)	42.68	44.23	46.74	48.87	43.84	45.77
F_3 (100%NP+75%K)	45.95	48.45	48.91	50.38	45.19	47.51
F_4 (100% NPK)	47.69	50.55	52.65	55.14	48.87	50.29
SEm±	0.62	0.73	1.51	1.59	1.21	0.98
C.D. (P=0.05)	1.94	2.25	4.65	4.86	3.74	2.88

Microbial strains						
S_1 (*Agrobacterium sp.*)	44.85	48.58	52.21	53.25	47.14	48.85
S_2 (*Flavobacterium sp.*)	38.84	40.10	43.98	45.12	42.54	42.91
S_3 (*Rhizobium sp.*)	40.10	42.69	45.82	47.68	44.19	44.83
S_4 (*Trichoderma sp.*)	42.75	44.99	47.07	48.91	46.15	46.34
SEm±	0.59	0.69	1.48	1.42	1.15	0.82
C.D.(P=0.05)	1.81	2.11	2.25	4.31	3.48	2.49

Actinomycetes population (cfu g^{-1} of soil)

The population of the actinomycetes showed higher colonies at 100%NP+75%K (F_3) and 100%NPK (F_4) which remained statistically at par with each other at all the stages of growth. The fertility level at F_3 on an average exhibited 9.43% more population than 100%NP+25%K (F_1) at 60 DAS.

The maximum actinomycetes population was observed at 60 DAS of baby corn growth. On an average *Agrobacterium sp*. (S_1) produced 18.36% more colonies than *Flavobacterium sp*. (S_2) – the least effective strain in increasing actinomycetes population. The overall effect of strains on the population was found to be in the order of S_1> S_4> S_3> S_2.

Table 4: Effect of microbial strains (KSB and *Trichoderma*) and fertility level on fungal population (colony forming unit) cfu/g of soil.

Treatments	Fungal population					
	Before sowing, 60DAS, After harvesting of baby corn					
	10^{-4} cfu/g		10^{-4} cfu/g		10^{-4} cfu/g	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
Fertility levels (RDF)						
F_1 (100%NP+25%K)	20.12	21.74	32.59	33.19	28.45	29.05
F_2 (100%NP+50%K)	24.52	26.23	35.77	36.55	31.63	32.41
F_3 (100%NP+75%K)	27.64	29.32	39.83	40.59	36.89	39.55
F_4 (100% NPK)	30.41	32.29	43.61	45.5	39.47	42.36
SEm±	0.92	1.06	1.23	1.61	0.88	0.91
C.D. (P=0.05)	2.97	3.41	3.84	4.99	2.71	2.86

Microbial strains						
S_1 (*Agrobacterium sp.*)	27.14	29.87	40.59	42.25	36.45	38.11
S_2 (*Flavobacterium sp.*)	20.35	21.56	32.68	33.08	28.54	30.94
S_3 (*Rhizobium sp.*)	22.47	23.95	35.09	36.68	31.05	32.54
S_4 (*Trichoderma sp.*)	24.86	26.12	36.92	38.03	33.72	35.79
SEm±	0.42	0.85	1.19	1.12	0.84	0.54
C.D.(P=0.05)	1.34	2.71	3.63	3.42	2.67	1.71

Fungal population (cfu $g^{-1}$of soil)

In comparison to total bacterial and actinomycetes, the fungal population was least and can be counted easily. The fertility level at 100%NP+75%K (F_3) while remaining at par with 100%NPK (F_4) exhibited higher no. of colonies at 10^{-4} dilution and remained statistically superior to other treatments. On an average F_3 registered 22.26% more fungal population than 100%NP+25%K (F_1) at 60 DAS of the crop growth.

The maximum fungal population was observed with *Agrobacterium sp*. (S_1) colonies at 60 DAS which was statistically significant over other treatments. It registered 25.97% more population than *Flavobacterium sp*. (S_2). Over all the effect was found in the order of S_1> S_4> S_3> S_2.

Table 5: Effect of microbial strains (KSB and *Trichoderma*) and fertility level on Soil microbial biomass carbon (SMBC) of soil.

Treatments	SMBC (µg C g^{-1} soil)					
	Before sowing of baby corn		60DAS		After harvesting of baby corn	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
Fertility levels (RDF)						
F_1 (100%NP+25%K)	73.39	76.52	98.65	99.12	90.85	92.36
F_2 (100%NP+50%K)	79.36	82.36	102.63	103.41	94.21	96.77
F_3 (100%NP+75% K)	92.74	96.32	119.86	120.92	98.32	99.47
F_4 (100% NPK)	94.23	100.15	124.85	126.11	102.12	103.88
SEm±	0.52	1.23	1.65	2.09	1.42	1.79
C.D. (P=0.05)	1.74	4.15	5.32	6.95	4.24	5.25
Microbial strains						
S_1 (*Agrobacterium sp.*)	92.63	94.36	120.87	122.65	100.65	101.41
S_2 (*Flavobacterium sp.*)	70.56	72.44	91.86	93.55	84.01	85.21
S_3 (*Rhizobium sp.*)	79.21	83.65	98.93	101.11	88.05	90.33
S_4 (*Trichoderma sp.*)	85.36	90.31	105.66	107.31	94.12	95.88
SEm±	0.56	1.16	0.99	1.76	0.62	1.78
C.D.(P=0.05)	1.74	3.59	3.19	5.63	1.98	5.41

Soil microbial biomass carbon was higher at 100%NP+75%K (F_3) and 100%NPK (F_4) which remained statistically at par to each other. On an average biomass carbon at F_3 was 17.86% higher than the 100%NP+25%K (F_1). Higher biomass carbon was observed at 60 DAS of crop growth and was in the order F_4, F_3> F_2> F_1.

SMBC was highest with *Agrobacterium sp*. (S_1) and this was followed by *Trichoderma sp*. (S_4) at all the stages of observation during both the years. On an average the biomass carbon by S_1 was 31.34% higher than the *Flavobacterium sp*. (S_2).

Result and discussion

Application of microbial inoculants or biofertilizer had a positive role in influencing the soil microbial population provided more of the nutrient in the second year in comparison to first year. The significant difference was observed with 100% NP+75%K and *Agrobacterium sp*., which remained at par with 100% NPK and *Agrobacterium sp*. followed and *Trichoderma sp*. The application of biofertilizer (KSB) and *Trichoderma* saves around 25% of the applied chemical fertilisers which could be considered as an alternative practice that improves soil health thereby fertility status of the soil and quality [Malik *et. al*., (2000); Sharma *et al*., (2014)] [10, 11].

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