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Effect of Sulphur and Rhizobium Inoculation on Rhizosphere and Non-rhizosphere Microflora

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Abstract : *Field and pot experiments on sulphur application with Rhizobium inoculation in relation to rhizospheric and non-rhizospheric microflora indicated that application of elemental sulphur upto 40 kg S ha^{-1} significantly increased the bacterial population. However, sulphur upto 60 kg S ha^{-1} decreased fungal and actinomycete population in rhizosphere and non-rhizosphere soils. Rhizobium inoculation with USDA - 3463 and M_4, significantly increased the microbial population over control. Further, the bacterial, fungal and actinomycete populations increased from 15 to 30 days after sowing and flowering stage , and thereafter decreased upto 60 days (harvesting stage).* (**Key words :** *Sulphur, Rhizobium, USDA - 3463, M_4, rhizosphere, non-rhizosphere)*

Sulphur being an important element present both in soil and living cells, is utilised by microorganisms for the synthesis of various organic S-compounds which govern the metabolic processes, growth, and multiplication. The soil microorganisms, by virtue of their key role in soil fertility, are affected by inoculated microbes and other amendments leading to reflections on fertility potential of soil. Long term application of elemental sulphur (S) reduced microbial activity in agricultural soils (Gupta *et al.* 1986). Maynard *et al.* (1986) showed the adverse effects of elemental sulphur deposition on microbial activity in forest soil. In view of this, it is imperative to study the effect of sulphur along with *Rhizobium* inoculation on total bacteria, fungi and actinomycetes in rhizosphere and non-rhizosphere soils.

MATERIAL AND METHODS

Field experiments with greengram for rhizosphere and pot experiments without crop for non-rhizosphere were conducted during *Kharif* 1991 and 1992 at Banaras Hindu University, Varanasi. The treatments for field experiments consisted of 4 levels of elemental sulphur (0, 20, 40 and 60/kg S ha^{-1}) and two strains of Rhizobium (USDA-3463 and M_4). There were 12 treatment combinations replicated 3 times in Factorial Randomized Block Design (Gomez & Gomez 1984).

Pot experiments consisted of 4 levels of elemental sulphur (0, 20, 40 and 60 kg S ha^{-1}) with 5 replications in Randomized Block Design. The soil samples in both the experiments were collected at 15, 30, 45 and 60 days after sowing (DAS). The rhizosphere and non-rhizosphere microflora, (total bacteria, fungi and actinomycetes) were estimated by plate count method on Asparagine Mannitol agar medium, Rose-bengal streptomycin agar medium and Kenknight and Munaier's medium respectively.

RESULTS AND DISCUSSION

Sulphur and Rhizobium Inoculation : The bacterial population increased significantly with an application of sulphur upto 40 kg S ha^{-1} (Table 1) while fungal and actinomycete populations decreased upto a dose of 60 kg S ha^{-1} in rhizosphere and non-rhizosphere soils at all stages during both the years (Table 2 and 3). The reduced populations of fungi and actinomycetes indicate possible changes in microbial interactions in sulphur treated plots. Significant changes in bacterial and fungal populations were observed due to short and long term acidification of SO_2 deposition (Bryant *et al.* 1979). Bewley and Perkinson (1984, 1985) also reported decreased

TABLE 1. Effect of S and Rhizobium on periodical changes in bacterial population (x $10^5 g^{-1}$ soil)

Sulphur treatments kg ha^{-1}	Kharif 1991				Kharif 1992			
	Days after sowing (DAS)				Days after sowing (DAS)			
	15	30	45	60	15	30	45	60
Field experiment with greengram (Rhizosphere)								
S_0	8.24	24.99	21.65	9.12	8.69	25.49	20.71	10.23
S_{20}	7.48	21.57	16.36	5.81	7.74	23.37	16.67	7.87
S_{40}	9.74	28.31	25.89	13.21	10.45	28.38	24.64	15.48
S_{60}	7.29	24.56	23.94	11.57	7.43	24.95	20.97	13.67
LSD (p=0.05)	1.31	2.12	0.93	1.12	1.47	1.58	1.58	1.08
Pot experiment without greengram (Non-rhizosphere)								
S_0	6.40	17.13	17.85	6.26	8.41	20.07	18.48	8.17
S_{20}	5.82	15.26	14.82	4.36	7.52	17.37	16.49	8.84
S_{40}	8.18	20.42	19.47	7.32	9.41	21.74	20.91	11.22
S_{60}	8.40	16.18	18.50	8.39	5.37	18.83	22.80	9.67
LSD (p=0.05)	1.65	1.84	1.30	0.55	1.42	1.39	0.67	2.06
Rhizobium inoculation								
Rh_0*	7.66	23.34	20.92	8.99	8.12	24.63	20.09	10.87
M_4	7.99	23.64	21.19	10.33	8.83	24.34	20.34	12.34
USDA-3463	8.91	27.61	23.76	10.15	9.11	27.67	21.83	12.83
LSD (p=0.05)	N.S	1.84	0.81	N.S	N.S.	1.37	1.37	0.94
Sulphur x Rhizobium inoculation								
S_0 x Rh_0 *	6.12	20.09	14.79	4.17	6.35	21.95	15.72	6.25
S_0 x M_4	8.07	21.66	16.39	7.07	8.42	23.27	17.05	9.47
S_0 x USDA-3463	8.24	22.97	17.92	6.18	8.46	24.88	17.24	7.90
S_{20} x Rh_0	11.74	24.66	21.85	7.57	10.62	25.97	21.27	9.24
S_{20} x M_4	5.82	23.72	19.28	10.60	7.46	23.57	19.02	11.20
S_{20} x USDA-3463	7.15	26.58	23.81	9.18	8.41	26.92	21.83	10.26
S_{40} x Rh_0	8.65	25.40	24.37	14.14	10.10	26.63	23.51	15.62
S_{40} x M_4	10.95	27.25	25.13	12.34	11.82	27.74	24.46	14.59
S_{40} x USDA-3463	9.63	32.29	28.00	13.15	9.44	30.76	25.95	16.22
S_{60} x Rh_0	4.13	23.19	22.67	10.07	5.40	23.97	19.84	12.38
S_{60} x M_4	7.10	21.91	23.97	10.60	6.77	22.78	20.83	13.65
S_{60} x USDA-3463	10.63	28.59	25.17	12.80	10.13	28.11	22.24	14.98
LSD (p=0.05)	2.27	N.S	1.62	2.24	2.55	N.S	N.S	1.87

* uninoculated

TABLE 2. Effect of S and Rhizobium on fungal population (X $10^3 g^{-1}$ soil).

Sulphur treatments kg ha^{-1}	Kharif 1991				Kharif 1992			
	Days after sowing				Days after sowing			
	15	30	45	60	15	30	45	60
Field experiment with greengram (Rhizosphere)								
S_0	5.88	21.95	15.60	5.63	6.63	23.08	15.31	7.46
S_{20}	3.20	19.45	14.17	4.98	5.51	22.56	15.89	6.94
S_{40}	3.10	17.27	11.52	3.77	4.98	20.53	13.50	5.67
S_{60}	1.62	12.43	9.08	2.20	3.94	15.65	11.97	4.10
LSD (p=0.05)	0.51	1.10	1.36	0.51	0.95	1.97	1.42	1.27
Pot experiment without greengram (Non-rhizosphere)								
S_0	3.12	19.00	14.39	4.17	4.60	19.50	17.26	6.09
S_{20}	2.30	15.45	12.24	3.14	3.59	16.97	14.47	4.16
S_{40}	2.11	10.32	9.22	1.73	2.45	12.89	10.10	2.80
S_{60}	1.04	8.57	6.41	1.40	1.80	9.46	8.93	1.76
LSD (p=0.05)	0.24	1.84	1.11	0.41	0.65	1.71	1.40	0.86
Rhizobium inoculation								
Rh_0 *	3.00	17.03	12.13	3.94	4.65	19.38	13.83	5.94
M_4	3.47	17.76	12.36	4.25	5.24	20.92	14.18	5.97
USDA-3463	3.89	18.53	13.29	4.26	5.91	12.07	14.49	6.22
LSD (p=0.05)	0.44	0.96	N.S.	N.S	0.83	N.S.	N.S.	N.S.
Sulphur x Rhizobium inoculation								
S_0 x Rh_0	4.07	23.64	16.60	6.12	5.01	24.98	17.09	7.53
S_0 x M_4	6.75	20.31	14.61	5.28	7.08	22.25	13.98	7.35
S_0 x USDA-3463	6.83	21.87	15.59	5.50	7.79	22.00	14.85	7.51
S_{20} x Rh_0	4.49	18.42	13.04	5.52	4.38	19.57	14.53	7.64
S_{20} x M_4	3.08	19.67	14.31	4.38	5.40	23.23	16.98	6.31
S_{20} x USDA-3463	2.04	20.26	15.16	5.04	6.74	24.88	16.16	6.87
S_{40} x Rh_0	2.06	15.63	10.64	3.06	5.83	18.78	12.95	5.50
S_{40} x M_4	3.02	18.31	11.45	3.85	5.09	21.86	13.25	5.09
S_{40} x USDA-3463	4.21	17.88	12.47	4.41	4.03	20.95	14.29	6.42
S_{60} x Rh_0	1.36	10.42	8.22	1.06	3.37	14.18	10.76	3.09
S_{60} x M_4	1.03	12.76	9.08	3.47	3.38	16.32	12.50	5.12
S_{60} x USDA-3463	2.47	14.12	9.95	2.08	5.06	16.46	12.66	4.08
LSD (p=0.05)	0.88	1.90	N.S.	N.S.	1.65	3.43	N.S	N.S.

* uninoculate

TABLE 3. Effect of S and Rhizobium on periodical changes in actinomycetes population (x $10^4 g^{-1}$ soil)

Sulphur treatments kg ha^{-1}	Kharif 1991				Kharif 1992			
	Days after sowing				Days after sowing			
	15	30	45	60	15	30	45	60
Field experiment with greengram (Rhizosphere)								
S_0	9.77	32.20	27.81	12.61	10.55	29.91	27.67	15.39
S_{20}	9.01	29.70	25.50	10.60	10.74	29.32	25.25	11.71
S_{40}	6.27	26.08	22.76	8.90	7.42	27.08	22.46	9.47
S_{60}	6.18	23.15	20.02	7.40	7.99	23.02	21.43	8.32
LSD (p=0.05)	1.98	2.40	2.39	0.85	1.31	2.98	2.88	1.52
Pot experiment without greengram (Non-rhizosphere)								
S_0	8.37	20.49	14.32	7.33	10.03	21.85	18.48	7.52
S_{20}	5.92	15.45	12.63	6.28	6.15	20.20	15.62	6.37
S_{40}	4.16	9.91	8.78	5.21	4.96	16.14	10.52	5.61
S_{60}	1.78	8.30	6.72	4.16	3.29	12.97	9.30	4.23
LSD (p=0.05)	1.25	1.12	0.52	0.53	1.15	1.14	1.20	0.41
Rhizobium inoculation								
Rh_0*	7.08	27.23	23.35	9.60	8.19	26.40	23.85	11.31
M_4	7.75	27.74	23.83	9.89	8.88	27.33	23.92	11.35
USDA-3463	8.60	28.38	24.88	10.14	10.46	28.26	24.84	11.01
LSD (p=0.05)	N.S	N.S	N.S	N.S	2.13	N.S	N.S	N.S
Sulphur x Rhizobium inoculation								
S_0 x Rh_0	10.52	35.22	30.24	12.45	11.03	32.08	29.08	15.62
S_0 x M_4	8.05	29.66	25.32	13.01	8.42	28.33	26.27	16.13
S_0 x USDA-3463	10.73	31.71	27.87	12.36	12.19	29.31	27.69	14.43
S_{20} x Rh_0	7.60	27.89	24.26	10.34	8.57	26.31	24.34	11.63
S_{20} x M_4	10.94	30.19	26.03	10.26	12.21	29.96	25.14	11.55
S_{20} x USDA-3463	8.50	31.01	26.21	11.20	11.43	31.69	26.76	11.95
S_{40} x Rh_0	4.76	24.14	20.07	9.18	6.06	25.27	21.82	10.41
S_{40} x M_4	6.16	26.98	23.98	8.90	7.09	27.91	22.76	9.81
S_{40} x USDA-3463	7.90	27.13	24.23	8.62	9.10	28.05	22.83	8.82
S_{60} x Rh_0	5.44	21.66	18.84	6.43	7.08	21.95	20.18	7.56
S_{60} x M_4	5.83	24.14	20.00	7.38	7.79	23.12	21.52	8.53
S_{60} x USDA-3463	7.26	23.66	21.21	8.39	9.11	24.00	22.58	8.85
LSD (p=0.05)	N.S	N.S	N.S	N.S	2.27	N.S	N.S	N.S

* Uninoculated

fungal biomass in SO_2 polluted soils. Significant reduction in the population of predatory protozoa that feed on fungi in sulphur treated plots were also observed by Gupta and Germida (1988). The results of present investigation are also in agreement with the findings of Gupta *et al.* (1988).

Bacterial, fungal and actinomycete populations were increased from 15 to 30 days after sowing and flowering stage (Table 1, 2 and 3). It might be reflected firstly, proper moisture supplied.through irrigation as reported by Mishustin (1954) who noted increase in microbial population in plant rhizosphere at 30-40 per cent moisture, and secondly due to high rate of metabolic activity of plants and also high amount of root exudation resulted in overall favourable rhizosphere effects. Inoculation with USDA-3463 or M_4 strain increased the bacterial and fungal population at all stages. Brown *et al.* (1962) were also of the opinion that the inoculation of soils, seeds and roots with *Rhizobium* resulted in an establishment of high number of microbes in rhizosphere of plants. Increase in *Azotobacter* counts due to inoculation of combined culture (*Rhizobium*+ *Azotobacter*) was reported by Rawat and Sanoria (1978).

Influence of Rhizosphere : Rhizosphere favoured higher bacterial, fungal and actinomycte populations at all stages (Table 1, 2 and 3). It was probably due to the secretion of beneficial metabolites from roots resulting in the activation of microbial activities leading to even greater availability of nutrient elements from the soil. It has been reported that plants release a variety of carbon compounds in their root exudates, the nature and quantities of which vary according to plant species (Rovira, 1959; Vancura & Hanzalikova 1972) and with plant age (Barber & Martin 1976). Root exudate release has been directly corelated with microbial biomass (Ritz & Robinson 1988). Different root exudates are selective for growth of certain microorganisms, and therefore, the higher microbial counts in rhizosphere as compared to non-rhizpsphere was due to the positive influence of root exudates on bacteria, fungi and actinomycetes.

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