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Studies on Certain Factors Influencing Legume-Rhizobia Interrelationship. II. Total Microbial Population in the Rhizosphere Region *

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The interrelationship between the altered rhizosphere microflora and rhizobia in the rhizospheres of green gram, black gram and sunnhemp following foliar spray with gibberellic acid (50 and 100 ppm), 2,4-dichlorophenoxy acetic acid (5 and 10 ppm) and indole acetic acid (250 and 500 ppm) was assessed. A positive correlation existed between the bacterial and rhizobial populations in the rhizophere of the three plant species. However, no such correlation was apparent between fungal and *Rhizobium* population in black gram while a positive relationship in green gram and a negative correlation in sunnhemp was evident. The interaction between actinomycetes and rhizobia revealed a positive influence in green gram and black gram while such a relationship did not exist in sunnhemp.

The ecology of host and nodule bacteria rhizobia adequately in natural and agricultural habitats has not been understood (Masefied, 1957; Petrosyan, 1959). That the rhizosphere region could be influenced by various environmental factors and physiological conditions of the plant has been reported by workers all over the world. It is well documented that foliar application of chemicals is one of the important methods of bringing about altered rhiozosphere effect (Katznelson, 1965). The present communication reports the establishment of rhizobia in the rhizosphere of green gram (*Phaseolus aureus* Roxb.), black gram (*P. mungo* L.) and sunnhemp (*Crotalaia juncea* L.) in relation to the quantitative variations in the populations of bacteria, fungi and actinomycetes brought about by foliar application of growth regulators.

MATERIALS AND METHODS

Ten surface sterilized seeds were sown in pots having garden land soil whose moisture was adjusted every alternate day to 60 per cent level of maximum water holding capacity. When the plants were 15 days old, the following spray treatments were given: (i) gibberellic acid (GA) (50 ppm and 100 ppm), (ii) 2, 4-dicholorophenoxy acetic acid (2, 4-D) (5 and 10 ppm) and (iii) indole acetic acid (IAA) (250 and 500 ppm).

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Spraying was done at weekly interval till 50th day in green gram and black gram; for sunnhemp the spraying was continued till 70th day. Rhizosphere soil samples for analysis were collected on 24th, 38th and 52nd days for green gram and black gram to represent respectively the vegetative, flowering and pod bearing phases of the crops. For sunnhemp the samples were taken on 38th, 52nd and 72nd day to represent the above stages.

The rhizosphere soil samples were collected as per the procedure described by Rangaswami and Balasubramanian (1963). While soil extract agar was used for the enumeration of bacteria and actinomycetes, Martin's rose bengal agar was used for fungi. The most probable number of *Rhizobium* was estimated from the serially diluted soil suspension by the plant dilution count method (Clark, 1965).

RESULTS AND DISCUSSION

The results obtained in the present study with green gram, black gram and sunnhemp confirm the findings of earlier workers in that the chemical sprays applied on the foliage subtly altered the rhizosphere microflora. The spray of GA-50 ppm and 2, 4-D-5 ppm on green gram and black gram and GA-100 ppm and 2, 4-D-10 ppm on sunnhemp, in general, increased the total bacterial population and also the population of *Rhizobium* sp. On the other hand, GA-100 ppm and 2,4-D-10 ppm decreased the total bacterial population and also the population of *Rhizobium* sp. as well in green gram rhizosphere (Table I, II). A positive correlation between the population of bacteria and that of rhizobia in the rhizosphere of the three plant species sprayed with growth regulators was discernible (Table V).

TABLE I. Changes in the *Rhizobium* population in the rhizospheres of green gram and sunnhemp as influenced by foliar spray of growth regulators (population in 10^6/g of moisture free soil)

	Green gram			Black gram			Sunnhemp		
	VS	FS	PS	VS	FS	PS	VS	FS	PS
GA — 50 ppm	10.12	42.89	31.42	9.61	17.78	12.28	2.90	4.69	6.78
GA — 100 ,,	0.35	7.76	4.61	7.86	23.32	15.20	15.87	13.34	14.66
2, 4-D — 5 ,,	6.10	30.15	11.25	0.92	13.35	16.26	12.81	23.72	20.25
2, 4-D — 10 ,,	0.72	5.66	3.60	1.85	9.92	9.54	20.94	18.43	11.71
IAA — 250 ,,	0.74	9.94	7.46	7.76	10.05	6.28	3.19	9.43	18.65
IAA — 500 ,,	6.92	19.76	16.10	12.55	17.72	12.66	14.74	8.57	12.18
Control (Distilled water spray)	2.73	15.26	21.27	3.81	8.14	3.11	6.93	14.17	11.63
Soil	0.32	0.40	0.35						

C.D. (P=0.05) Stage : 2.18 Treatments : 3.32 Between concentration within growth regulators : 3.32

VS-Vegetative stage; FS-Flowering stage; PS-Pod bearing stage.

TABLE II. Changes in the bacterial population in the rhizospheres of green gram, black gram and sunnhemp as influenced by foliar spray of growth regulators (Population in 10^4/g of moisture free soil)

Treatment	Green gram			Black gram			Sunnhemp		
	VS	FS	PS	VS	FS	PS	VS	FS	PS
GA — 50 ppm	130.74	751.69	361.66	97.36	298.56	205.21	111.82	332.82	240.60
GA — 100 ,,	9.71	38.72	65.28	62.04	348.39	297.67	197.45	691.95	482.51
2, 4-D — 5 ,,	63.10	168.20	121.21	77.38	120.16	161.00	63.42	413.20	374.67
2, 4-D — 10 ,,	84.60	69.41	57.29	460.84	228.81	212.07	225.46	530.46	540.54
IAA — 250 ,,	140.87	152.72	126.71	96.18	127.40	118.71	207.91	194.69	547.88
IAA — 500 ,,	218.98	199.10	222.54	117.48	146.00	211.74	88.07	343.43	308.24
Control (Distilled water spray)	102.62	81.29	76.24	147.27	118.76	91.54	84.13	297.14	241.06
Soil	8.09	6.02	7.25						

C.D. (P=0.05)		Control *Vs* growth regulators	28.19
Crops	: 21.30	Between growth regulators	23.01
Stages	: 21.30	Between concentrations within growth regulators	32.54
Treatment	: 32.54		

VS-Vegetative stage; FS-Flowering stage; PS-Pod bearing stage

TABLE III. Changes in the fungal population in the rhizospheres of green gram, black gram and sunnhemp as influenced by foliar spray of growth regulators (population in 10^4/g of moisture free soil)

Treatment	Green gram			Black gram			Sunnhemp		
	VS	FS	PS	VS	FS	PS	VS	FS	PS
GA — 50 ppm	13.18	19.29	17.61	9.92	8.57	12.43	10.78	8.59	14.21
GA — 100 ,,	8.33	12.60	14.84	12.55	8.81	11.86	12.74	8.94	9.73
2, 4-D — 5 ,,	11.61	19.92	6.43	6.65	5.03	8.74	12.48	10.11	7.88
2, 4-D — 10 ,,	19.74	16.37	4.91	18.34	5.18	11.39	11.10	4.40	5.20
IAA - — 250 ,,	12.76	16.69	18.27	1.54	10.03	8.41	31.62	7.35	18.44
IAA - — 500 ,,	12.39	20.35	24.26	2.59	20.45	14.72	11.62	9.40	21.52
Control (Distilled water spray)	20.28	20.51	13.27	9.38	22.55	15.20	9.39	6.54	11.75
Soil	5.41	3.91	4.56						

C.D. (P=0.05)	Crops : 1.16	Treatments	1.77	Control *Vs* growth regulators	1.53
				Between growth regulators	1.25

VS-Vegetative Stage, FS-Flowering Stage, PS-Pod bearing Stage

Fungal population, in general, decreased due to various spray treatments in the rhizospheres of green gram and black gram; however, GA and IAA sprays increased them in sunnhemp rhizosphere (Table III). The inhibition of rhizobia by the culture filtrates of fungi has been reported earlier (Nillson, 1957; Holland, 1962; Holland and Parker, 1966) while Hattingh and Louw (1966) observed that fungi isolated from clover seeds had very little effect on the growth of rhizobia tested. In the present study though no correlation existed between the fungal and *Rhizobium* populations in the rhizo-

sphere of black gram, a positive correlation in sunnhemp were observed (Table V) indicating thereby that the influence of the rhizosphere fungi on the *Rhizobium* population in the root region varied with plant species. Perhaps, the qualitative nature of the rhizosphere fungi influences the proliferation or suppression of *Rhizobium* in the rhizosphere as observed in the three legume species.

There was a general decrease in actinomycete population due to the spray of growth regulators at vegetative stage in green gram and black gram, while in sunnhemp a general increase was noticed (Table IV). A positive correlation was observed between the actinomycete and *Rhizobium* populations in the rhizosphere of green gram and black gram (Table V).

TABLE IV. Changes in the actinomycete population in the rhizospheres of green gram, black gram and sunnhemp due to foliar spray of growth regulators (population 10^6/g of moisture free soil)

Treatment	Green gram			Black gram			Sunnhemp		
	VS	FS	PS	VS	FS	PS	VS	FS	PS
GA — 50 ppm	3.04	2.30	6.65	0.47	4.29	4.68	0.28	1.90	3.88
GA — 100 ,,	0.23	2.08	10.21	0.26	3.91	5.97	0.08	1.81	7.21
2, 4-D — 5 ,,	1.72	4.61	2.74	0.69	0.14	1.29	0.86	1.89	2.05
2, 4-D — 10 ,,	0.66	0.73	0.66	1.20	0.09	1.54	0.92	4.80	3.79
IAA — 250 ,,	0.87	2.72	1.76	0.28	6.25	8.20	2.48	1.98	5.05
IAA — 500 ,,	0.75	4.76	7.85	0.46	8.41	12.11	0.72	1.41	9.40
Controlled (Distilled water spray)	2.50	1.78	3.85	1.19	1.08	3.09	0.47	2.48	7.58
Soil	0.63	0.90	0.82						

C.D. (P=0.05) Stages : 0.41 Treatments : 0.63 Control *VS* growth regulators : 0.56
Between growth regulators : 0.46 Between concentrations within growth regulators : 0.63
VS-Vegetative Stage, FS-Flowering Stage, PS-Pod bearing Stage

TABLE V. Correlation between the rhizosphere bacteria, fungi and actinomycetes and rhizobia

Independent variable	Dependent variable	Coefficient of correlation 'r'	Coefficient of regression 'b'	Prediction equation
1. Green gram		**		
Bacterial population	*Rhizobium*	0.79	0.06	Y : 0.056 X + 3.3
Fungal ,,	population	0.40	0.90	Y : 0.90 X − 1.73
Actinomycete ,,	,,	0.36**	1.58	Y : 1.58 X + 7.43
2. Black gram				
Bacterial population	,,	0.30*	0.02	Y : 0.02 X + 7.51
Fungal ,,	,,	0.07 NS	—	—
Actinomycete ,,	,,	0.32*	0.54	Y : 0.54 X + 8.76
3. Sunnhemp				
Bacterial population	,,	0.31*	0.01	Y : 0.01 X + 9.44
Fungal ,,	,,	0.27*	−0.26	Y : −0.26 X +15.71
Actinomycete ,,	,,	0.13 NS	—	—

** Significant at 1% level, * Significant at 5% level, NS-Not significant

These results, thus, suggest that *Rhizobium* population in the rhizosphere of the legume plants might be influenced by the other microflora in that region and that such an effect varied with the plant species.

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