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Effect of Trace Elements on Groundnut *Rhizobium*

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Under *in vitro* conditions, the trace elements at lowest concentrations (Cobalt 0.5 ppm, copper 1.0 ppm, manganese 1.0 ppm, zinc 1.0 ppm and molybdenum 1.0 ppm) caused 50 to 300 per cent more growth of groundnut rhizobium. Copper at 5.0 and 10.0 ppm level inhibited the growth effectively. The two-way combination of trace elements enhanced the growth of rhizobium significantly; manganese and molybdenum caused the maximum growth. The copper toxicity to the rhizobium seems to be alleviated by the presence of other trace elements like manganese, molybdenum, zinc and cobalt.

The role of trace elements in plant nutrition is well documented. It is well known that *Rhizobium* population in soil is influenced by many physical, chemical and biotic factors of soil (Hallsworth, 1958; Nutman, 1963; Fahraeus and Ljunggren, 1968). The effect of cobalt under *in vitro* conditions and of molybdenum in soils in relation to rhizobial growth were reported (Lowe and Evans, 1962). However, the influence of trace elements either individually or in combination on the growth of rhizobia was not studied. The present paper deals with the influence of trace elements on rhizobia as reflected by their growth under *in vitro* conditions.

MATERIALS AND METHODS

Fifty ml of yeast extract mannitol broth (without calcium carbonate) were dispensed into an Erlenmeyer flask. Five trace elements viz., cobalt, copper, manganese, molybdenum and zinc either individually or in two-element combinations at three dtfferent doses were prepared and added to the broth. The flasks were then sterilized in an autoclave at 20 psi. After cooling one ml of cell suspension of *Rhizobium* sp. from *Arachis hypogaea* multiplied in yeast extract mannitol broth over 48 hr, was added and incubated at room temperature (28 ± 1°C) on a rotary shaker operating at 250 rpm. The growth rate of *Rhizhobium* was measured after 12, 24, 48, 96 and 108 hr of incubation in a Spectronic 20 spectrophotometer at 600 nm.

RESULTS AND DISCUSSION

The presence of individual trace elements in the medium had varying effects on the growth of *Rhizobium*. When compared to the control, all the trace elements at the lowest concentration caused better growth of the organism. However, cobalt and copper inhibited the growth for 24 to 48 hr and thereafter the organism adjusted to the

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TABLE I. Effect of trace elements on the growth of *Rhizhobium*
(Data represent the OD values)

		Incubation period (hr)				
Treatment	Dose (ppm)	12	24	48	96	108
Control (No trace element)	—	0.020	0.025	0.025	0.025	0.030
Cobalt	0.5	0.032	0.040	0.045	0.055	0.100
	2.5	0	0.010	0.045	0.045	0.045
	5.0	0	0.010	0.010	0.010	0.010
Copper	1.0	0.020	0.065	0.065	0.070	0.110
	5.0	0	0.015	0.020	0.020	0.030
	10.0	0	0.015	0.015	0.015	0.015
Manganese	1.0	0.035	0.065	0.070	0.075	0.090
	10.0	0.045	0.070	0.075	0.075	0.120
	20.0	0.040	0.070	0.095	0.095	0.110
Zinc	1.0	0.045	0.065	0.080	0.085	0.110
	5.0	0.250	0.055	0.055	0.060	0.100
	10.0	0.005	0.060	0.060	0.065	0.110
Molybdenum	1.0	0.040	0.065	0.065	0.065	0.080
	2.0	0.040	0.070	0.070	0.075	0.090
	3.0	0.035	0.060	0.060	0.065	0.070

medium and exhibited growth. Copper at 5.0 and 1.0 ppm level was inhibitory througout the period of study (Table I). The present results are in agreement with the earlier results of Hallsworth *et al.* (1960) who reported that Cu at high level (6.4ppm) was lethal to Rhizobium while at lower levels (0.064 to 0.64 ppm) there was significant increase in the growth and nitrogen content. Vincent (1954) stated that Cu might be toxic to rhizobial growth if direct contact between the bacterium and the element is encountered and this view supdorts the present finding.

The effect of trace elements in two-way combinations on the growth of *Rhizobium* is presented in Tables II and III. The two-way combination of trace elements had a considerable effect on the multiplication of rhizobia. The combination of manganese and molybdenum enhanced the growth of this bacterium. The significance of addition of Mo to the soil for the full expression of symbiotic association of a legume-rhizobium association has been stressed by Vincent (1968). Since Anderson (1942) observed stimulation of clovers by wood ash and found this to be due to Mo, a very large volume of

TABLE II. Effect of combinations of trace elements on the growth of *Rhizobium*

(Data represents the OD values)

Treatment	Incubation period (hr) 12	24	48	96	108
Control (No trace element)	0.020	0.025	0.025	0.025	0.030
Cobalt + Copper					
Co 0 5 ppm + Cu 1.0 ppm	0.020	0.060	0.060	0.065	0.090
Co 2.5 ppm + Cu 5.0 ppm	0	0.010	0.010	0.015	0.015
Co 5.0 ppm + Cu 10.0 ppm	0	0.015	0.015	0.015	0.025
Cnbalt + Manganese					
Co 0.5 ppm + Mn 1.10 ppm	0.035	0.070	0.070	0.070	0.100
Co 2.5 ppm + Mn 10.0 ppm	0	0.030	0.060	0.065	0.110
Co 5.0 ppm + Mn 20.0 ppm	0	0.010	0.050	0.055	0.130
Cobalt + Zinc					
Co 0.5 ppm + Zn 1.0 ppm	0.060	0.120	0.135	0.160	0.210
Co 2.5 ppm + Zn 5.0 ppm	0	0.040	0.050	0.055	0.085
Co 5.0 ppm + Zn 10 ppm	0	0.010	0.050	0.060	0.080
Cobalt + Molybdenum					
Co 0.5 ppm + Mo 1.0 ppm	0.030	0.070	0.070	0.070	0.100
Co 2.5 ppm + Mo 2.0 ppm	0	0.010	0.010	0.020	0.050
Co 5.0 ppm ÷ Mo 3.0 ppm	0	0.010	0.015	0.015	0.050
Copper + Manganese					
Cu 1.0 ppm + Mn 1.0 ppm	0.035	0.055	0.070	0.080	0.100
Cu 5.0 ppm + Mn 10.0 ppm	0	0.005	0.010	0.010	0.020
Cu 10.0 ppm + Mn 20.0 ppm	0	0.010	0.025	0.080	0.070
Copper + Zinc					
Cu 1.0 ppm ÷ Zn 1.0 ppm	0.060	0.060	0.060	0.040	0.040
Cu 5.0 ppm + Zn 5.0 ppm	0	0.010	0.015	0.035	0.020
Cu 10.0 ppm + Zn 10.0 ppm	0	0.010	0.010	0.025	0.035
Copper + Molybdenum					
Cu 1.0 ppm + Mo 1.0 ppm	0.035	0.060	0.060	0.065	0.090
Cu 5.0 ppm + Mo 2.0	0.020	0.030	0.040	0.040	0.030
Cu 10.0 ppm + Mo 3.0 ppm	0.020	0.010	0.015	0.010	0.035

TABLE III. Effect of combinations of trace elements on growth of *Rhizobium*
(Data represent the OD values)

Treatment	Period of inocubation (hr)				
	12	24	48	96	108
Control (No trace element)	0.020	0.025	0.025	0.025	0.020
Manganese + Zinc					
Mn. 1.0 ppm + Zn 1.0 ppm	0.030	0.080	0.080	0.100	0.120
Mn. 10.0 ppm + Zn 5.0 ppm	0.035	0.070	0.080	0.090	0.100
Mn. 20.0 ppm + Zn 10.0 ppm	0.030	0.080	0.100	0.110	0.130
Manganese + Molybdenum					
Mn. 1.0 ppm + Mo. 1.0 ppm	0.040	0.060	0.065	0.080	0.100
Mn. 10.0 ppm + Mo. 2.0 ppm	0.045	0.060	0.065	0.100	0.120
Mn. 20.0 ppm + Mo. 3.0 ppm	0.065	0.200	0.210	0.260	0.340
Zinc + Molybdenum					
Zn. 1.0 ppm + Mo. 1.0 ppm	0.040	0.060	0.070	0.080	0.035
Zn. 5.0 ppm + Mo. 2.0 ppm	0.020	0.040	0.060	0.070	0.100
Zn. 10.0 ppm + Mo. 3.0 ppm	0.025	0.070	0.070	0.080	0.110

work has been done in Australia on the importance of this element in permitting efficient nitrogen fixation in the potentially effective nodules. Though not much information is available on the effect of Mn on rhizobial growth, its importance can well be understood by referring to some work carried out with other nitrogen fixing organisms. Becking (1961) observed that the growth and nitrogen fixing ability of *Azotobacter chroococcum* was considerably increased by the presence of Mn in the environment. Further, *Beijerinckia* was known to grow well even in soil containing a high Mn indicating the adaptability of this organism to high doses of Mn. Kesslor *et al* (1957) suggested that Mn is important in O_2 evolving system. It is plausible to think that *Rhizobium* being and aerobic and heterotrophic organism, might well be influenced by the combination of Mo and Mn.

It is also observed that Zn in combination with Mn, Co or Mo significantly increased the growth of *Rhizobium*. Betrand and De Wolf (1959) showed that *Aspergillus niger* required Zn for the synthesis of tryptophan. Tryptophan is one of the precursors of indole acetic acid (IAA) and the fact that IAA plays an important role in the curling of root hairs and subsequent nodulation has been recognised by many workers (Kefford *et al.*, 1960; Nutman, 1965). The present study suggests that Zn status in soil in combination with elemements like Mn, Co or Mo might influence the *Rhizobium* population and eventually the nodulation of the legumes.

The combination of Cu with other elements like Co, Mo, Mn, however, significantly increased the growth of bacterium when compared to the control. This may, perhaps, be due to the alleviation of Cu toxicity by the presence of other elements. The effect of different doses of elements on the multiplication of rhizobia revealed that while the lower dosage enhanced the growth, the higher doses had varying effects, the reasons for such variation await further study.

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