Proc. Indian natn. Sci. Acad. B45 No. 6 pp 639-642 (1979)

Influence of Trace Elements on Growth of Rhizobia and on Nodulation in *Trifolium alexandrinum*

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(Received 16 August 1978)

Effect of four trace elements viz., zinc sulphate, molybdenum oxide, manganese sulphate and copper sulphate on growth of five different strains of *Rhizobium* was studied *in vitro* at 10, 50, 200 and 400 ppm. Copper sulphate inhibited the growth of all the test rhizobia at all concentrations while zinc sulphate did so at 50, 200 and 400 ppm. Molybdenum oxide and manganese sulphate were ineffective at all concentrations.

Effect of foliar spray of three trace elements viz., zinc sulphate, molybdenum oxide and manganese sulphate on nodulation of *Trifolium alexandrinum* was studied at 50, 100 and 150 ppm. Manganese sulphate increased the number of nodules marginally at 50 ppm but it decreased the nodule number and weight at 150 ppm. Zinc sulphate and molybdenum oxide reduced the nodule number significantly at 150 ppm. Zinc sulphate reduced the weight of nodules significantly at 150 ppm whereas molybdenum oxide reduced the weight as well as volume of nodules significantly at 100 and 150 ppm.

Key Words: Trace elements, Rhizobia, Nodulation, *Trifolium alexandrinum*

Introduction

Much work has been carried out on different aspects pertaining to the influence of trace elements on the physiology of different plants including legumes, but surprisingly little attention has been given to their possible influence on *Rhizobium* and on nodulation. The influence of trace elements on nodulation has been studied by some workers (Brenchley & Thornton 1925, Quellette & Dessureaux 1958, Vose and Jones 1963). Mulder and Van Veen (1960) have shown with *Trifolium pratense* that nodulation may be quite ineffective on acid soils which contain toxic levels of certain trace elements. The present study deals with the effect of four trace elements viz., zinc sulphate, molybdenum oxide, manganese sulphate and copper sulphate on the growth behaviour of five different strains of rhizobia. The effect of foliar spray of three trace elements viz., zinc sulphate, molybdenum oxide and manganese sulphate on nodule number, weight and volume of *Trifolium alexandrinum* was also studied.

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Materials and Methods

Five different *Rhizobium* strains viz., *R. trifolii*, *R. phaseoli* strain 1, *R. phaseoli* strain 2, *R. leguminosarum*, *R.* sp. (cowpea group) were isolated from the effective nodules of *Trifolium alexandrinum*, *Phaseolus mungo*, *P. aureus*, *Pisum sativum* and *Vigna catjang* respectively. The rhizobia were maintained on yeast mannitol agar medium.

A qualitative study was made by streak method to note the effect of four trace elements viz., zinc sulphate, molybdenum oxide, manganese sulphate and copper sulphate on the growth behaviour of above-mentioned rhizobia. Different concentrations viz., 10, 50, 200 and 400 ppm of trace elements were prepared separately, added in sterile cooled (40°C) yeast mannitol agar medium so as to maintain the respective concentrations. The medium was shaken well and poured into sterile Petri dishes. It was allowed to solidify. The streaks were made on the solidified agar discs from the individual strains of *Rhizobium*. Three replicates were prepared for each concentration. The streaks made on the yeast mannitol agar discs without trace elements served as control. Petri dishes were incubated at 25°C±(1) for 48 hr and thereafter the results were recorded.

Foliar spray of zinc sulphate, molybdenum oxide and manganese sulphate was studied as follows: Seeds dressed with effective strain of *Rhizobium* were sown in earthenware pots containing equal amount of uniformly mixed unsterile soil. After a week, only five healthy seedlings were retained in each pot. Trace elements of 50, 100 and 150 ppm were prepared in distilled water. The plants were sprayed with the help of home-spray atomizer separately with each concentration. First spraying was done when the plants were 15 days old followed by another spraying after 5 days of first spraying. The control plants were sprayed with distilled water only. Plants were watered regularly to maintain the moisture content. After 20 days of second spraying, the plants were dug out gently and the root system was washed in running water. The nodules were detached from the roots, counted, dried in folds of blotting paper and weighed. The volume of nodules was determined by dipping the known number of nodules in a known quantity of water in a graduated measuring cylinder. The weight and volume/100 nodules were recorded for comparison.

Results and Discussion

Amongst the trace elements assayed, copper sulphate inhibited the growth of all the test rhizobia at all concentrations while zinc sulphate did so at 50, 200 and 400 ppm. Molybdenum oxide and manganese sulphate were ineffective (table 1). Findings of the present observations with regard to the effect of copper and zinc on growth of *Rhizobium* strains are in agreement with those of Allington (1945) and Kernkamp (1948).

Amongst the trace elements sprayed, zinc sulphate and molybdenum oxide reduced the number of nodules significantly at 150 ppm. Zinc sulphate reduced the weight of nodules significantly at 150 ppm whereas molybdenum oxide reduced the weight and volume of nodules significantly at 100 and 150 ppm (table 2). Manganese sulphate increased the number of nodules marginally at 50 ppm but with an increase in its concentration i.e. 150 ppm, the number as well as weight of nodules decreased significantly. The reason for increase or decrease in nodule number, weight and volume of treated plants may be due to the changes in metabolic activities of plants. Combined volume of infected nodule cells has been correlated as one of the quantitative character with effectiveness of

Table 1 In vitro *studies on effect of trace-elements on growth of rhizobia*

Test trace-element	Concentration (in ppm)	*Rhizobium trifolli*	*R. legumi-nosarum*	*R. phaseoli* strain 1	*R. phaseoli* strain 2	*Rhizobium* sp. (cow pea group)
	10	+	+	+	+	+
Zinc sulphate	50	—	—	—	—	—
	200	—	—	—	—	—
	400	—	—	—	—	—
Molybdenum oxide	10	+	+	+	+	+
	50	+	+	+	+	+
	200	+	+	+	+	+
Manganese sulphate	400	+	+	+	+	+
	10	+	+	+	+	+
	50	+	+	+	+	+
	200	+	+	+	+	+
	400	+	+	+	+	+
Copper sulphate	10	—	—	—	—	—
	50	—	—	—	—	—
	200	—	—	—	—	—
	400	—	—	—	—	—
Control	—	+	+	+	+	+

\+ Indicates growth of rhizobia
— Indicates inhibitory effect

Table 2 *Effect of foliar spray of trace elements on nodulation in the test plant*

Trace element sprayed	Concentration (in ppm)	No. of nodule/plant (mean of 5 values)	't' value	Wt./100 nodule (mean of 5 values) (in g)	't' value	Volume/100 nodule (mean of 5 values) (in ml)	't' value
	50	129.0	0.775	0.311	1.204	0.300	0.661
Zinc sulphate	100	116.08	2.154	0.284	2.346	0.283	2.060
	150	114.00	2.789**	0.226	3.628*	0.283	2.060
	50	131.66	0.701	0.314	1.020	0.333	2.120
Molybdenum oxide	100	116.08	1.987	0.148	5.864*	0.183	5.80*
	150	74.33	7.475*	0.121	7.363*	0.166	9.166*
	50	154.00	3.016**	0.376	0.185	0.40	0.875
Manganese sulphate	100	126.3	1.389	0.336	0.682	0.283	2.060
	150	114.30	2.783**	0.186	3.472*	0.283	1.350
Control	—	138.00	—	0.364	—	0.386	—

*Significant at 1% level
**Significant at 5% level

nodulation in peas, clover and soybeans (Chen & Thornton 1940). Nodule volume and weight were significantly correlated with the eventual harvest of dry weight of clover plants (Jones 1961). In the present investigations the total number, weight and volume of nodules were reduced with certain concentration of trace elements and such reductions may therefore also influence the effectiveness of symbiosis in the test plants.

Acknowledgement

The authors are thankful to the Head of Botany Department for providing necessary facilities.

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