

## ZINC EFFECT ON NITROGEN FIXATION AND CLUSTERBEAN YIELD

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### ABSTRACT

Nitrogenase activity of nodules, carbohydrate and protein contents of leaves and nodules were studied in clusterbean in response to zinc application. Zinc significantly increased the nitrogenase activity, carbohydrate and protein contents. At 60 days the contents of protein, carbohydrate and nitrogen were higher than at 80 days. There was some parallelism in total nitrogenase activity and nitrogen content per plant. The seed yield response to zinc as reflected in the number of pods has been discussed in relation to nitrogen fixation and dry matter production. Spray with  $ZnSO_4$  proved the most effective treatment in this regard.

### INTRODUCTION

Clusterbean is a multipurpose kharif legume, in arid and semi-arid zones of India for food, fodder and industrial purposes. The crop is poorly manured due to uncertain weather conditions and lack of information on proper doses of nutrient elements. Most of work done on the nutrition of this crop has been related with major elements (Dalal and Tomer 1978; Gill and Singh 1981; Dahiya et al. 1986), whereas the significance of micronutrients is still ignored. Therefore, this experiment was designed keeping in view earlier findings on the use of different forms of zinc and its mode of application. This information would be valuable for large areas of Haryana under zinc deficient conditions.

### MATERIAL AND METHODS

The plants of clusterbean CV HG 75 were raised in pots containing 5 kg of washed river sand. Zinc in the form of  $ZnSO_4$  and ZnO with concentration of 5 kg/ha and 10 kg/ha was applied separately by thorough mixing with sand. Before flowering, three foliar sprays of 0.5%  $ZnSO_4$  were made at fortnightly intervals. Treatment of ZnO was also used for seed coating of 50 g/kg and 100 g/kg of seed. Effective *Rhizobium* culture was given in the form of broth immediately before sowing. At weekly intervals, plants were supplied with nitrogen free nutrient solution (Wilson and Reisenauer 1963). In control plants, no zinc was applied. Samples were drawn

at initiation of flowering and 50% pod formation (60 and 80 days after sowing respectively). The plants were removed from the pots by gently shaking and tapping of the sand.

Nitrogenase activity of nodules was assessed by Acetylene Reduction Assay (Hardy et al. 1968) using Gas Chromatograph (Nucon 5700, Hydrocarbon Analyser). Total soluble carbohydrates and total soluble protein of nodules and leaves were estimated following Yemm and Willis (1954) and Lowry et al. (1951) respectively. Extraction was done according to the method of Barnett and Naylr (1966). Micro Kjeldahl's technique (A.O.A.C. 1970) was followed to determine the nitrogen content of nodules. Grain yield was recorded at maturity and the data were analysed statistically.

## RESULTS AND DISCUSSION

More than double increase in seed yield was observed with 0.5% ZnSO<sub>4</sub> spray followed by 100 g/kg seed coating of ZnO as compared to zinc deficient plant. Similarly the same treatments gave the highest number of pod/plant.

Application of zinc increased the total dry weight of the plant when sampled at 60 and 80 DAS (Table 1). The increase in seed yield by zinc application was from 37.6% to 133% in respect of an enhancement in dry matter. Thus, the changes in growth ultimately increased the seed yield through the acceleration of metabolic activities specially of protein, carbohydrates and nitrogen fixation.

Table 1. Effect of Zinc on dry matter and yield of clusterbean

Treatment	Dry wt (g) per plant		No. of pods per plant	Seed wt (g) per plant
	60 days	80 days		
Control	0.368	1.344	6.25	0.664
<b>Zinc Sulphate (ZnSO<sub>4</sub>)</b>				
5 kg/ha	0.598	1.909	9.75	1.154
10 kg/ha	0.900	2.333	7.50	1.066
<b>Zinc Oxide (ZnO)</b>				
5 kg/ha	0.830	1.744	7.30	0.915
10 kg/ha	0.521	1.723	7.75	1.043
<b>Seed treatment (ZnO)</b>				
50 g/kg seed	0.720	1.420	6.60	1.005
100 g/kg seed	0.604	1.520	8.75	1.102
0.5% spray of ZnSO <sub>4</sub>	1.079	2.780	11.80	1.450
C D 5%	0.182	0.294	1.02	0.312

Protein contents of nodules and leaves were higher at 60 DAS as compared to 80 DAS. Zinc treatments significantly increased the protein content of nodules and leaves (Table 2). The resultant increase in protein content was possibly due to increased nitrogen fixation by nodules (Table 3). Decline in protein content in leaves and nodules at 80 DAS appeared to be due to the translocation of the fixed nitrogen at formation of flowers and pods to the active sinks.

Table 2. Effect of zinc on total soluble protein content ( $\text{mg g}^{-1}$  fr. wt.)

Treatment	Nodules		Leaves	
	60 days	80 days	60 days	80 days
Control	62.30	45.26	63.43	52.94
<b>Zinc sulphate (<math>\text{ZnSO}_4</math>)</b>				
5 kg/ha	89.03	55.07	65.39	56.00
10 kg/ha	76.45	49.29	83.62	61.68
<b>Zinc Oxide (<math>\text{ZnO}</math>)</b>				
5 kg/ha	81.25	55.33	80.80	56.72
10 kg/hg	84.00	57.84	73.56	62.20
<b>Seed Coating (<math>\text{ZnO}</math>)</b>				
50 g/kg seed	79.19	49.16	79.09	71.94
100 g/kg seed	87.27	48.12	67.46	63.46
0.5 % spray of $\text{ZnSO}_4$	80.22	56.27	72.04	60.80
C D 5%	7.50	6.68	7.82	5.96

Application of zinc increased total soluble carbohydrate content significantly in leaves and nodules (Table 3). The maximum increase was observed with 0.5% spray of  $\text{ZnSO}_4$ . This increase is likely due to increase in photosynthetic surface area.

Table 3. Effect of zinc on total soluble carbohydrate content ( $\text{mg g}^{-1}$  fr. wt)

Treatment	Nodules		Leaves	
	60 days	80 days	60 days	80 days
Control	6.52	6.27	10.48	9.85
<b>Zinc Sulphate (<math>\text{ZnSO}_4</math>)</b>				
5 kg/ha	6.91	6.80	12.14	10.69
10 kg/ha	11.45	6.88	16.67	12.50
<b>Zinc Oxide (<math>\text{ZnO}</math>)</b>				
5 kg/ha	10.80	6.42	20.30	17.40
10 kg/ha	9.44	8.42	15.04	10.33
<b>Seed Coating (<math>\text{ZnO}</math>)</b>				
50 g/kg seed	10.51	9.06	18.12	10.69
100 g/kg seed	7.72	6.52	16.31	10.42
0.5% spray of $\text{ZnSO}_4$	14.20	6.96	22.47	13.77
C D 5%	2.13	1.48	2.02	2.33

The carbohydrate content declined at 80 DAS in both the organs possibly due to the translocation of assimilates to the developing pods and seeds.

Higher dose of zinc in any given form increased total nitrogenase activity of nodules (Table 4) and spray with 0.5% of  $ZnSO_4$  gave the highest activity which was more than 3 times of control. However, at 80 DAS, seed coating with ZnO was the most effective in this regard. The observations indicate that zinc application increased the size of the nodules which may contain more number of bacteroids as compared to control. However, increased nodulation under zinc application seems to be the major factor to enhance nitrogen fixation per plant.

The total nitrogenase activity at 80 DAS was high but the total nitrogen content ( $mg\ g^{-1}$ ) of nodules was low, and reverse was true at 60 DAS (Table 4). This decrease in nitrogen content of nodules could be due to fast utilization of fixed nitrogen by the plant for developing pods and seeds, and also for stored as protein. The total nitrogenase activity showed some rough parallelism with nitrogen content of nodules per plant with time interval. Zinc application brought about better nodulation, high nitrogenase activity, and increased nitrogen content of nodules significantly. Prasad and Ram (1982) reported a similar increase in total nitrogen content in mungbean and Kapur et al. (1975) in soybean plants.

Table 4. Total nitrogenase activity, nitrogen content of nodules and leaf area as affected by zinc

Treatment	Nitrogenase activity ( $\mu\text{ mol C}_2\text{H}_4\text{ plant}^{-1}\text{ hr}^{-1}$ )		Nitrogen content ( $\text{mg g}^{-1}$ dry wt.)		Leaf area ( $\text{cm}^2$ )
	60 days	80 days	60 days	80 days	80 days
Control	3.56	5.08	12.97	8.80	58.15
<b>Zinc Sulphate (<math>ZnSO_4</math>)</b>					
5 kg/ha	4.07	6.68	17.71	12.68	88.19
10 kg/ha	5.19	6.89	16.80	13.53	101.90
<b>Zinc Oxide (ZnO)</b>					
5 kg/ha	8.07	8.88	18.55	11.70	103.64
10 kg/ha	9.47	9.24	20.47	14.08	101.85
<b>Seed Coating (ZnO)</b>					
50 g/kg seed	8.09	12.61	18.75	13.73	95.55
100 g/kg seed	8.49	13.53	16.80	13.95	75.61
0.5% Spray of $ZnSO_4$	11.74	9.33	19.50	13.94	123.92
C D 5%	2.71	2.01	4.60	2.12	10.84

## REFERENCES

- A.O.A.C. 1970. Official Methods of Analysis of the Association of Official Agricultural Chemists, Washington, D.C.
- Barnett, N.M. and Naylor, A.W. 1966. Amino acid and protein metabolism in bermuda grass during water stress. *Plant physiology* 41: 1222-1230.

- Dahiya, S.S., Khera, A.P., Tomar, N.K., Grewal, K.S. and Panwar, B.S. 1986. Response of fertilizers to guar under irrigation constraints. *Guar Research Annals* 4: 18-20.
- Dalal, S.K. and Tomer, P.S. 1978. A note on the comparative efficiency of guar (*Cyamopsis tetragonoloba* (L.) Taub.) varieties with varying phosphorus levels and seedling rates on quality of the forage. *Annals of Arid Zone* 17: 253-254.
- Gill, P.S. and Singh, K. 1981. Effect of fertilization on yield contributing characters and grain yield of clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.) varieties. *Haryana Agricultural University Journal of Research* 11: 333-338.
- Hardy, R.W.F., Halstan, R.D., Jackson, E.K. and Burns, R.C. 1968. The acetylene/ethylene assay for nitrogen fixation: Laboratory and field evaluation. *Plant Physiology* 43: 1185-1207.
- Kapur, O.C., Gangwar, M.S. and Tilak, K.V.B.R. 1975. Influence of zinc on symbiotic nitrogen fixation by soybean (*Glycin max* Linn.) in silt to loam soil. *Indian Journal of Agricultural Research* 9: 51-56.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with folin phenol reagent. *Journal of Biological Chemistry* 193: 265-275.
- Prasad, J and Ram, H. 1982. Variation in varietal response to rhizobium and zinc in mungbean (*Vigna radiata*). *Journal of the Legume Research* 5: 42-44.
- Wilson, D.O. and Reisnauer, H.M. 1963. Cobalt requirement of symbiotically grown alfalfa. *Plant and Soil* 19: 364-373.
- Yemm, E.W. and Willis, A.J. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal* 57: 508-514.