

RESEARCH ARTICLE

Effect of zinc, molybdenum and urea on chlorophyll and protein content of mungbean (*Vigna radiata* L. Wilczek)

■ KUSUM MALIK, SATISH KUMAR AND K.P. SINGH ARYA

SUMMARY

The effect of zinc, molybdenum and urea has been studied on chlorophyll, nitrogen and protein content of mungbean crop. Two varieties of mungbean [*Vigna radiata* (L.) Wilczek] i.e., Pant Mung-4 and Narendra-1 were studied in the Department of Botany, Meerut College, Meerut (U.P.) in the years 2011-2012. Simple Randomized Block Design was followed with 4 replications and 11 treatments along with control. The treatments were Zn (5, 10, 15 and 20 ppm), Mo (1, 2, 3 and 5 ppm) and urea (1% and 2%). The results were found significant of both varieties of mungbean.

Key Words : Zinc, Molybdenum, Mungbean (*Vigna radiata* (L.) Wilczek)

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The legumes specially mungbean and urdbean are being cultivated on marginal lands because of their deep root system and nodulation and nitrogen fixing capacity and have thus enjoyed the inputs of irrigation and fertilization.

Mungbean and urdbean are generally grown in summer and *Kharif* seasons in U.P. Area and production

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of pulse crops have been discussed by Kushwah and Nagar (2006). Mungbean and urdbean are the second largest protein producing pulse crop of the world where as soybean and groundnut rank first position. Since they contain 23-25 per cent protein in their grain, they could provide an answer to the problem of protein deficiency as well as protein malnutrition (Rosario *et al.*, 1980).

Considerable variation for productivity exists between important pulses growing countries in the region. In order to ensure "household nutritional security" as per recommendations of the International Conference on Nutrition (ICN), concerted efforts are needed at this stage to improve further and productivity of pulse crop in most of the pulses growing countries in Asia (Paroda, 1994).

Bio availability of zinc and molybdenum are poor. Zinc is one of the commonest micronutrient essential for plants growth. Plant roots absorb zinc as ion Zn^{2+} and as a component of synthetic and natural molecular complex. Soluble zinc salts and zinc complexes can also enter the plant system directly through leaves. Zinc is closely involved in the diversity of enzymatic activities and nitrogen metabolism of plants. In zinc deficient plant, protein synthesis and protein levels are markedly reduced and aminoacids and amides are accumulated.

Among the micronutrients the molybdenum has long been known to be required for normal assimilation of N_2 in plant. It is found in two enzymes, which occur in plants are xanthine oxidase and nitrate reductase. The nitrate reductase is found in all the plant species as well as fungi and bacteria. The enzyme is essential for assimilation of nitrates since it catalysis the first step of reduction of NO_3 to NH_3 . The other major molybde-protein of plants nitrogenase fixes N_2 to NH_3 which then assimilated by plants. It is composed of at least two proteins, both of which have as iron requirement and one of which has Mo requirement. It is supposed to be similar to nitrate reductase. Sarando and Asborn (1996) studied the effect of foliar urea spraying on biomass production, harvest index, grain yield and grain protein content on three rice cultivars. Nitrogen (30 kg/ha) was applied as foliar urea spraying at the end of tillering, heading or postanthesis spraying nitrogen at heading increased grain yield due to higher grain number/m² and a more efficient dry matter partition to the grain (harvest index) without changes in biomass production. In all the three cultivars N spraying at heading increased grain protein production per ha due to an increase of both grain yield and grain protein percentage. Nitrogen spraying in rice, even at low doses, could be effective to increase grain yield and grain protein content depending on rice cultivars and time of application. Effect of foliar spray of urea 1 and 2 per cent on the growth and yield of mungbean (*Vigna radiata* L.) has been studied in this research work.

Dwivedi *et al.* (1992) studied in trials with a blackgram (*Vigna mungo*)/wheat cropping sequence, application of 10 kg N/ha and 0.5 kg Mo/ ha and seed inoculation with *Rhizobium phaseoli* significantly increased seed yields and N, P, K, protein and essential amino acid contents and showed similar residual effect in wheat. Among N sources, urea was superior in the first year and compost in the second year. Superphosphate (SSP) and inoculation were superior in

increasing yields of both crops. The effects of urea were found significant in increasing seed/ grain protein and amino acid contents, whereas inoculation was superior to Mo application. Kalyanaraman and Sivagurunathan (1994) reported that *Vigna mungo* was given 0, 50, 100, 150, 200 or 250 mg Zn/kg soil. The leaves were analysed for aminoacids and chlorophyll. The extinction coefficient 'k' was calculated. Variations in 'k' values with leaf age and Zn conc. appear to vary linearly with the variations in essential amino acids content. The 'k' values increased with 100 mg Zn/kg soil and decreased at rates above 150 mg Zn. They further studied the effect of cadmium, copper and zinc on the growth of blackgram. Mevada *et al.* (2006) conducted a field experiment on sandy loam soil to study the effect of application of NPKS individually as well as with different levels of micronutrients (Zn, B, Mo and Fe) on the performance and economics of urdbean [*Vigna mungo* (L.) Hepper]. The maximum grain yield (1180 kg/ha) was obtained under chelated iron (1 kg/ha) + N-P-K-S (20-18-17-20 kg/ha) treatment with 21.6 per cent increase over control (924 kg/ha). The higher gross realization and net profit were recorded with the same treatment.

MATERIAL AND METHODS

The present trials were conducted at Meerut College, Meerut (U.P.) during the years 2011-2012. The seeds of mungbean Var. Pant Mung-4 and Narendra-1 were obtained from G.B. Pant University of Agricultural and Technology, Pantnagar (U. Singh Nagar) Utrtrakhand. The seeds were sown directly in the plots. R.B.D. was followed with 4 replications and 11 treatments. After 30 days of sowing the crop was sprayed with different concentrations of zinc, molybdenum and urea solutions.

Symbols of treatments :		
Sr. No.	Treatments	Symbols
1.	Control (c)	T ₁
2.	Zn 5 ppm	T ₂
3.	Zn 10 ppm	T ₃
4.	Zn 15 ppm	T ₄
5.	Zn 20 ppm	T ₅
6.	Mo 1 ppm	T ₆
7.	Mo 2 ppm	T ₇
8.	Mo 3 ppm	T ₈
9.	Mo 5 ppm	T ₉
10.	Urea 1%	T ₁₀
11.	Urea 2%	T ₁₁

Determination of total chlorophyll :

The chlorophyll content in fresh leaves of mungbean var. Pant Mung-4 and Narendra-1 was determined according to Arnon (1949) on 60th day of sowing. The procedure for chlorophyll determination was based on the work of Mac. Kinney (1941), on the absorption of light by aqueous acetone (80%) extracts of chlorophyll. Organic solvent, 4:1 acetone alcohol was used.

0.5g fresh leaves samples of control and treated plants were taken with organic solvent (Acetone 80%) in clean specimen tubes. The extracts were centrifuged at 3000 rpm for 15 minutes and the volume was made up to 25 ml of each sample by adding more organic solvent.

Spectra – 20 was used at Deptt. of Botany, Meerut College, Meerut (U.P.) and the observations of total chlorophyll content were recorded at 645, 652 and 663 wave lengths, respectively.

Total chlorophyll content was calculated by using the following formulae (Arnon, 1949).

$$C = 20.2 D_{645} + 8.02 D_{663} \text{ in mg/g dry weight.}$$

Estimation of total nitrogen and protein :

Nitrogen percentage and the amount of protein content synthesized by the tissues were determined according to Jackson (1958) and Misra (1968). 500 mg well dried and powdered plant material was taken in 50 ml Kjeldahl flask with 5 ml of H₂SO₄. 0.1g catalyst mixture of copper sulphate, potash sulphate and selenium dioxide in the ratio of 1:8:1, respectively was also added.

After digestion, the volumes were made upto 50 ml. Distillation was done in a Markham apparatus as described by Jackson (1958) and Misra (1968).

$$\text{Nitrogen percentage} = (T - B) \times 5 \times N \times \frac{1.4}{S}$$

where,

T = Volume of HCl (Standard acid used in actual titration)

B = Blank

N = Normality of HCl = N/10

1.4 = Constant

S = Dry weight of plant sample in g.

The difference (T-B) was multiplied by 5 because only 10 ml digested material out of 50 ml was distilled.

The protein content was determined by multiplying the total nitrogen by 6.25.

RESULTS AND DISCUSSION

The results regarding the effect of zinc, molybdenum and urea on chlorophyll content, nitrogen percentage and protein content of mungbean var. Pant Mung-4 and Narendra-1, respectively presented in Tables 1 and 2. The effect of zinc from T₂ to T₅ increased the chlorophyll, nitrogen and protein contents in both varieties of mungbean. The effect of molybdenum was found significant from T₆ to T₈ for chlorophyll, nitrogen and protein percentage. T₉ (5 ppm) showed toxic effect of molybdenum on chlorophyll, nitrogen and protein contents. 5 ppm Mo concentration ions increased the toxicity of mungbean plants *i.e.*, Pant Mung-4 and Narendra-1. The results of this finding are similar to the following research workers.

Kishor *et al.* (1991) studied the effect of iron and molybdenum nutrition on nodulation, symbiotic N fixation and grain yield of urdbean. *Vigna mungo* was given 0, 5 or 10 kg Fe and 0, 0.5 or 1 kg Mo/ha. Fe and Mo application increased number, DW and N content of nodules measured at 35 and 65 days after sowing. The highest yield per pot was obtained with 10 kg Fe and 0.5 kg Mo/ha.

Selvi and Ramaswamy (1995) reported that black gram (*Vigna mungo*) was grown on residual fertilizer after double cropped rice which received NPK fertilizer rates, ZnSO₄, S as CaSO₄, FYM and green leaf manure. Seed yield (638 kg/ha) was highest in plots receiving the recommended NPK fertilizer + 25 kg ZnSO₄. Masood and Mishra (2000) studied nutrient imbalance is one of the major abiotic constraints limiting productivity of pulses. The inbuilt mechanism of biological N₂ fixation enables pulse crops to meet 80-90 per cent of their N requirements, hence a small dose of 15-25 kg N/ha is sufficient to meet the requirement of most of the pulse crops. However, in new cropping systems like rice-chickpea, a higher dose of N (30-40 kg/ha) showed beneficial effect. Phosphorus deficiency is wide spread and most of the pulse crops have shown good response to 20-60 kg P₂O₅/ha depending upon nutrient status of soil, cropping system and moisture availability. Response to K application is location specific. In the recent years, use of 20-30 kg S/ha and some of the micronutrients such as Zn, B, Mo and Fe have improved productivity of pulse crops. Rao *et al.* (2003) studied the effects of ammonium sulphate, urea and ammonium nitrate at 2 and 3 per cent on blackgram. The fertilizers were sprayed during flower initiation and pod formation stages. Urea

at 3 per cent which was found superior among treatments, gave the highest number of pods per plant (14.90) and seed yield (8.25 q/ha), and significantly increased leaf and seed nitrogen, protein contents and 100 seed weight. Pavadai *et al.* (2004) reported that effects of different concentrations of Zn (zinc sulfate at 10, 25, 50, 100, 200 and 500 mg/lit.) on the seed germination, seedling growth and chemical composition of black gram cv. CO-3. The parameters tested were: germination percentage, root length, shoot length, root dry weight and shoot dry weight. The maximum root length, shoot length and dry weight were obtained under Zn at 10 mg/lit., while the minimum values were obtained at Zn at 500 mg/lit. The minimum and maximum chlorophyll a, b and total chlorophyll contents were obtained under 500 and 10 mg Zn/lit., respectively. Sugar and protein contents in the roots, shoots and leaves

also decreased with increasing Zn concentration. Malik (2004) conducted experiments at Research Farm of C.C.R. (P.G.) College, Muzaffarnagar to study the effect of Zinc, molybdenum and urea on the growth and yield of rice (*Oryza sativa* L.). Two varieties Saket-4 and Vardan were selected for the research work. Eleven treatments were considered for the study e.g. T₁ (control), T₂ (Zn 2.5 ppm), T₃ (Zn 5 ppm), T₄ (Zn 10 ppm), T₅ (Zn 15 ppm) T₆ (Mo 0.5 ppm), T₇ (Mo 1.0 ppm), T₈ (Mo 1.5 ppm), T₉ (Mo 2.0 ppm), T₁₀ (urea 1%) and T₁₁ (urea 2%). Significant effect of zinc T₄ and T₅, molybdenum T₇ and T₈ and of urea 2 per cent were found on morphological and biochemical characters of Rice (*Oryza sativa*). The main characters were plant height, number of tillers, leaf area, number of leaves, fresh weight and dry weight per plant, seed yield and 100 seed weight (g). Chlorophyll, nitrogen, and protein content

Table 1 : Effect of different concentrations of zinc, molybdenum and urea on chlorophyll content (mg/g dry weight) in mungbean on 60th day of sowing

Treatments	Pant Mung-4		Narendra-1	
	2011	2012	2011	2012
T ₁ (C)	26.2	26.8	25.6	25.7
T ₂	26.6	27.0	25.9	26.1
T ₃	26.9	27.3	26.3	26.7
T ₄	27.5	27.8	26.7	27.1
T ₅	28.0	28.5	27.5	27.8
T ₆	26.4	26.8	25.8	25.9
T ₇	27.2	27.4	26.4	26.8
T ₈	27.6	27.9	26.9	27.3
T ₉	27.1	27.2	26.3	26.5
T ₁₀	27.9	28.2	26.0	26.2
T ₁₁	28.8	29.1	27.0	27.2

Table 2 : Effect of different concentrations of zinc molybdenum and urea on nitrogen percentage and protein content in mungbean at harvesting stage

Treatments	Pant Mung-4				Narendra-1			
	2011		2012		2011		2012	
	Nitrogen %	Protein %	Nitrogen %	Protein %	Nitrogen %	Protein %	Nitrogen %	Protein %
T ₁	2.50	15.62	2.51	15.67	2.48	15.50	2.49	15.56
T ₂	2.51	15.67	2.52	15.75	2.49	15.56	2.51	15.67
T ₃	2.53	15.81	2.55	15.94	2.50	15.62	2.52	15.75
T ₄	2.56	16.00	2.59	16.19	2.52	15.75	2.53	15.81
T ₅	2.60	16.25	2.62	16.37	2.55	15.94	2.56	16.00
T ₆	2.52	15.75	2.53	15.81	2.50	15.62	2.50	15.62
T ₇	2.54	15.87	2.55	15.94	2.51	15.67	2.52	15.75
T ₈	2.57	16.06	2.58	16.12	2.54	15.87	2.55	15.94
T ₉	2.53	15.81	2.54	15.87	2.51	15.67	2.52	15.75
T ₁₀	2.59	16.19	2.61	16.31	2.57	16.06	2.59	16.19
T ₁₁	2.63	16.43	2.65	16.55	2.59	16.19	2.61	16.13

showed better results in comparison with control treatment. T₉ showed toxic effect. Sritharan *et al.* (2007) reported the physiological studies for yield enhancement in black gram (*Vigna radiata* L.). A trial was conducted to investigate the physiological and biochemical effects of foliar spray of chemicals and plant growth regulator on physiological characters and productivity of blackgram. The treatments consisting of foliar spray of diammonium phosphate, DAP 2 and urea 2 per cent, urea foliar spray (equivalent N content as in DAP) and control. Among all the treatments, 2 per cent urea had the profound effect in improving the total chlorophyll content, soluble protein content and NRase activity. Foliar sprays of 2 per cent urea recorded the highest grain yield of 955.20 kg/ha. The yield enhancement may be due to the improved morphological, physiological, biochemical and yield parameters *viz.*, plant height, number of pods per plant, grain yield, harvest index, chlorophyll content, soluble protein content and nitrate reductase activity. Singhal *et al.* (2007) conducted studies to investigate the effects of different Zinc (Zn) rates (5.3, 6.3, 7.3, 8.3 and 9.3 mg/lit.) and combined effect of Zn, Mg and sucrose on growth of 2 black gram cultivars (PU-35 and T-9). A concentration dependent decrease in plant height, fresh weight and chlorophyll, carbohydrate and protein contents as well as nitrate reductase activity was observed in both cultivars. However, proline content increased with increase in Zn rates. All the rates of combined treatments (Zn with Mg and sucrose) were able to alleviate reduction caused by Zn, but the alleviation was more pronounced with Mg supplementation than sucrose. Meenu (2010) studied the effect of zinc, molybdenum and urea on the growth, yield, chlorophyll content, nitrogen percentage and protein content in urdbean. All characters showed better performance in comparison to control. T₅ (Zn) showed best results for chlorophyll and protein content and also T₈ (Mo) showed best performance for all the above characters. T₉ (Mo) *i.e.*, 5 ppm showed toxic effect on urdbean plants in PU-31 and PU-30 varieties.

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