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## **Studies on the Impact of Micronutrient (Molybdenum) on Germination, Seedling Growth and Physiology of Bengal Gram (*Cicer arietinum*) under Laboratory Condition**

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### **ABSTRACT**

The present investigation deals with the study of impact of micronutrient towards growth, development and physiology of crop plants under varying conditions ranging from deficiency to excess. Under laboratory condition, the impact of different levels of molybdenum on germination, seedling growth and other physiological attributes of Bengal gram (*Cicer arietinum*) were studied. *Cicer arietinum* seeds were selected and sown in Petri dish with different treatment solutions except control. Each treatment was replicated thrice. Morphological, growth and biochemical attributes of gram seedlings were measured during the experimental period. The present investigation showed that, there is significant ( $p < 0.05$ ) increase in % germination for all the treatments. Fresh weight and dry weight of gram seedlings significantly ( $p < 0.05$ ) increased up to 2 ppm molybdenum concentration and then reduced. Significant ( $p < 0.05$ ) increase in the level of sugar, chlorophyll were recorded up to 7.5 ppm of molybdenum treatment and for ascorbic acid it is up to 6 ppm. From the enzyme assays, significant reduction ( $p < 0.05$ ) of peroxidase and nitrate reductase activity and enhanced catalase activity were observed at higher concentration of molybdenum treatment. From the Scanning electron microscopic study it was found that molybdenum has pronounced effect on the structural organization of root, shoot and leaves.

**Key words:** Molybdenum, bengal gram, scanning electron microscopy, chlorophyll, catalase activity

### **INTRODUCTION**

Molybdenum (Mo) is an essential trace element for most organisms as it occurs in more than sixty enzymes catalyzing diverse oxidation reduction reactions. This element has a crucial role in plant nitrogen metabolism being involved in the process of nitrogen fixation, nitrate reduction and the transport of nitrogen in plants (Marschner, 1995; Hamlin, 2007).

Application of micronutrients, yeast, thiamine and riboflavin has shown great activity on increasing growth and yield productivity of flax cv. Giza 5 (El-Shahawy *et al.*, 2008). All plants and animals require nutrients in order to maintain their life cycles. This discriminatory application of micronutrients to high value crops may be partly due the fact that their nutrient requirements have been more extensively studied (Rahman *et al.*, 2007). Genetic enhancement of crop cultivars with elevated levels of these micronutrients would be cost effective sustainable way of solving global micronutrient malnutrition problem (Velu *et al.*, 2011). Application of micro nutrients significantly affected all the growth traits of sugarcane variety CP-65/357, whereas plant height, tops weight,

cane length, internodes and length of internodes were significantly increased by the application of all the micro nutrients over the control (Jamro *et al.*, 2002). Application of different micronutrient solutions significantly influenced seedling emergence and early growth, fresh and dry weight of roots and shoots (Arshad Ullah *et al.*, 2002).

Micronutrients are elements essential for plant growth which are needed in very small quantities. It has molecular weight of 95.94 and atomic number 42. It stays in soil as molybdate form ( $\text{Mo O}_4^{2-}$ ). Plants uptake molybdenum as molybdate ions. Molybdenum is a cofactor of enzymes such as nitrate reductase, nitrogenase etc. Phosphate ions enhances molybdenum uptake in plants (Bambara and Ndakidemi, 2010).

*Cicer arietinum* (scientific name of gram) an important pulse crop in India, has the ability to grow under nitrogen deficient condition and because of its symbiotic association with *Rhizobium* which form nodules in the plant roots and binds free nitrogen from the air to meet the nitrogen requirement of the plant (Zohary and Hopf, 2000). *Cicer arietinum* is an annual herbaceous plant with a life span of 4-5 months attaining maximum height of 50 cm. Certain attributes viz., rapid growth and uniform germination rates renders the suitability of this crop plant species towards short term experiment on laboratory and long term experiments in the field. Higher molybdenum concentration was inhibitive towards growth and development of plants. The molybdenum deficiency symptoms include deep chlorosis of old leaflets (middle portion), spreading to young growth and intensification of chlorosis leading to bleaching. Affected leaves of *Cicer arietinum* were found to be dried and withered (Nautiyal and Chatterjee, 2004).

Spinach plants grown under deficient condition of molybdenum revealed lower leaf nitrate reductase activity and yield with respect to control (where adequate level of molybdenum is present). Molybdenum shows differential response towards different enzyme systems and processes associated with the plant body. Therefore, the nature of plant response to molybdenum deficiency seems to be complex. Molybdenzymes involved in nitrogen metabolism often leads to overall reductions in plant growth and health, which therefore, hampers plant development, susceptibility to pest damage and fruit and grain development (Graham and Stangoulis, 2005).

Some studies suggested that the physiological functions of molybdenum had two aspects. First, molybdenum performed physiological functions as an important composition of enzymes. Second, it could change physiological and biochemical characteristics of enzymes through changing the important participated substances of enzymes (Liu and Yang, 2001). The aim of present study was to assess the impact of different molybdenum dose on germination, seedling growth and physiology of gram seedlings under laboratory conditions.

## MATERIALS AND METHODS

To study the effects of different levels of molybdenum on germination, seedling growth and other physiological attributes an experiment were conducted in the laboratory of Department of Environmental Science, the University of Burdwan, West Bengal, India during March to June, 2009. Seeds of Bengal gram (*Cicer arietinum*) were collected from Directorate of Agriculture, Government of West Bengal and the seeds were kept in airtight packets at room temperature and were used as experimental materials. In the present investigation, the source of molybdenum was sodium molybdate salt. The treatment concentration viz, T<sub>1</sub>-1.5 ppm, T<sub>2</sub>-2 ppm, T<sub>3</sub>-2.5 ppm, T<sub>4</sub>-3 ppm, T<sub>5</sub>-4 ppm, T<sub>6</sub>-4.5 ppm, T<sub>7</sub>-5 ppm, T<sub>8</sub>-6 ppm, T<sub>9</sub>-7.5 ppm, T<sub>10</sub>-8 ppm and T<sub>11</sub>-10 ppm were prepared from stock solution (1000 ppm) along with a control (T<sub>12</sub>). After collection of seeds, the seeds were surface sterilized with 0.1% HgCl<sub>2</sub> for 30 sec and then washed with fresh water, followed by

distilled water. Twenty healthy and uniform sized seeds were selected and sown at equal distance in a Petri dish (9 cm dia) lined with filter paper moistened with different treatment solutions except control. The experiment was done in randomized block design having three replications. The entire set up was kept in a germination cage. In each day interval the entire set up was observed and treated with respective solution.

The rate of seed germination was recorded for every 24 h up to five days International Seed Testing Association (ISTA, 1976). The Petri dishes were covered with a net and kept in a growth room under optimum temperature and light condition. The length of shoot and root were recorded by using a centimeter scale from 10 days old seedlings. For fresh weight 10 days old seedlings were collected and soaked in blotting paper for removal of excess water and the final weight was recorded. The dry mass of shoot and root was recorded from 10 days old seedlings after keeping them in an oven at 80°C for 72 h.

For biochemical estimation of Total chlorophyll (Arnon, 1949), sugar (McReady *et al.*, 1950), Ascorbic acid (Mukherjee and Choudhury, 1983) from leaves were standard methods were followed.

For assay of Peroxidase activity (Chance and Maehly, 1955), Catalase Activity (Lacoppe and Gaspar, 1968), nitrate reductase activity (Hageman and Hucklesby, 1971) standard methods were followed.

For the study of histology of plant parts, plant parts (leaves, shoot and root) were fixed in 2.5% glutaraldehyde solution and then passed through alcohol grades and the photograph were taken through Scanning Electron Microscope (SEM) (Glauert and Lewis, 1998; Robards and Wilson, 1993; Allen and Lawrence, 1994-96; Hayat, 2000).

**Statistical analysis:** The results were analyzed by Analysis of Variance (ANOVA), with treatments as the independent variable. All statistical analysis were carried out with the program SPSS 11.0 for windows) All values were expressed as mean values Mean comparison were conducted using Fisher's Least Significant Differences (LSD) test following Cochran and Cox (1959) and Panse and Sukhatme (1967).

## RESULTS

### Growth attributes

**% germination:** In the present investigation the % germination was found to be high both under control and treated condition. For every treatment it was upto 100% with some few exceptions (Table 1).

**Root length and shoot length:** Higher root and shoot length was recorded in case of molybdenum treated plants in comparison to control. From treatment T<sub>1</sub> to treatment T<sub>6</sub> Root length and Shoot length of molybdenum treated plants gradually increased but from treatment T<sub>7</sub> it decreased. Treatment T<sub>6</sub> showed the highest shoot length (235.6 cm) and root length (167.3 cm), respectively. Lowest shoot length (130.75 cm) and root length (52.7 cm) were recorded for treatment T<sub>1</sub>, respectively (Table 1).

**Fresh weight and dry weight:** Fresh weight and dry weight of gram seedlings were found to be significantly higher in molybdenum treated plants with respect to control. Fresh weight of gram seedlings ranged between 0.075 g (T<sub>12</sub>) to 0.350 g (T<sub>2</sub>). Highest fresh weight was recorded in treatment T<sub>2</sub>. From treatment T<sub>2</sub> onwards a declining trend was found in fresh weight of gram

Table 1: Germination and growth attributes of gram seedlings under various concentration of molybdenum treatment

Treatments	% Germination	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Dry weight (g)
T <sub>1</sub>	100	52.7	130.75	0.147	0.053
T <sub>2</sub>	95	95.7	137.3	0.350	0.141
T <sub>3</sub>	100	130	145.35	0.315	0.043
T <sub>4</sub>	100	165.4	182.95	0.254	0.052
T <sub>5</sub>	98	167.1	227.3	0.248	0.057
T <sub>6</sub>	100	167.3	235.6	0.266	0.101
T <sub>7</sub>	100	142.3	216.20	0.237	0.076
T <sub>8</sub>	100	124.4	211.6	0.173	0.050
T <sub>9</sub>	100	136.56	214.1	0.188	0.046
T <sub>10</sub>	100	137.4	240.6	0.178	0.065
T <sub>11</sub>	100	128.1	233.5	0.184	0.049
T <sub>12</sub>	100	118.9	226.5	0.075	0.047
SEM (±)	0.936	2.195	2.387	0.003	0.003
CD (5%)	2.408	3.688	3.845	0.128	0.132
CV	0.567	0.662	0.483	13.795	47.485
LSD (5%)	0.944	1.446	1.508	0.050	0.052

SEM: Standard Error Mean's = Critical difference, CV: Covariance, LSD: Least Significant Difference between treatment means

Table 2: Photosynthetic pigment content in leaves of gram seedlings under various concentrations of molybdenum treatments

Treatments	Chlorophyll "a" (mg g <sup>-1</sup> fw)	Chlorophyll "b" (mg g <sup>-1</sup> fw)	Total chlorophyll (mg g <sup>-1</sup> fw)
T <sub>1</sub>	0.025	0.055	0.198
T <sub>2</sub>	0.013	0.042	0.167
T <sub>3</sub>	0.013	0.045	0.179
T <sub>4</sub>	0.060	0.060	0.242
T <sub>5</sub>	0.063	0.065	0.254
T <sub>6</sub>	0.083	0.070	0.266
T <sub>7</sub>	0.083	0.073	0.279
T <sub>8</sub>	0.086	0.083	0.286
T <sub>9</sub>	0.093	0.090	0.348
T <sub>10</sub>	0.083	0.084	0.158
T <sub>11</sub>	0.073	0.076	0.216
T <sub>12</sub>	0.010	0.064	0.178
SEM(±)	0.003	0.003	0.005
CD (5%)	0.137	0.131	0.168
CV	56.077	45.673	17.023
LSD(5%)	0.054	0.051	0.066

SEM: Standard Error Mean's = Critical difference, CV: Covariance, LSD: Least Significant Difference between treatment means

seedlings. Dry weight value of gram seedlings ranged between 0.047 g (T<sub>12</sub>) to 0.141 g (T<sub>2</sub>). Highest dry weight was recorded in treatment T<sub>2</sub> (Table 1).

### Biochemical study

**Chlorophyll a, b and total chlorophyll:** Higher level of chlorophyll-a, b and total chlorophyll in leaves were recorded in molybdenum treated plants in comparison to control (Table 2). The chlorophyll content in leaves increased unto treatment T<sub>9</sub> and then decreased gradually. The significant and higher results for chl-a (0.093 mg g<sup>-1</sup> fw), chl-b (0.090 mg g<sup>-1</sup> fw) and total chl (0.348 mg g<sup>-1</sup> fw) were obtained for treatment T<sub>9</sub> (Table 2).

**Sugar content:** Sugar content in different plant parts significantly increased upto treatment T<sub>9</sub> and then subsequently reduced. Highest accumulation of sugar was found to be in treatment T<sub>9</sub> (Table 3).

There is variable rate of accumulation of ascorbic acid in different plant parts. Treatments T<sub>6</sub>, T<sub>7</sub>, T<sub>8</sub> showed significant and higher accumulation of ascorbic acid in root (5% for T<sub>6</sub>; 5.03% for T<sub>7</sub> and 10.06% for T<sub>8</sub> respectively), shoot (1.57% for T<sub>6</sub>; 1.78% for T<sub>7</sub> and 1.99% for T<sub>8</sub> respectively) and leaf (4.05% for T<sub>6</sub>; 5.78% for T<sub>7</sub> and 7.60 % for T<sub>8</sub>, respectively) in comparison to other treatments. Higher accumulation was found to be in case of root and leaves (Table 3).

**Nitrate reductase, catalase and peroxidase activity:** From the leaf enzyme assay it was found that nitrate reductase activity significantly reduced at higher concentration of molybdenum treatment. Highest activities were recorded in treatment T<sub>6</sub> (12.60 mg/g/h). Catalase and peroxidase activity showed antagonistic nature under different treatment levels of molybdenum (Fig. 1).

**Scanning electron microscopic (SEM) study:** From the scanning electron micrograph plant roots of gram seedlings without molybdenum treatment shows normal occurrence of xylem and phloem in separate patches arranged on alternate radii. Xylem is exarches i.e., protoxylem is directed towards the periphery and metaxylem towards the centre. Xylem and phloem patches are intervened by small amount of parenchyma cells called conjunctive tissue. In mature stage, phloem is capped by thick walled sclerenchymatous tissue (Fig. 2). Plant shoot shows distinct structures of xylem and phloem. Metaxylem and protoxylem was arranged properly with normal vessels. Cambium layer is also prominent (Fig. 3). Leaves of untreated plants showed distinct mesophyll cells along with distinct palisade and spongy parenchyma. Distinct stomata with stomatal opening are presented in Fig. 4.

Table 3: Biochemical constituents in different plant parts under molybdenum treatments

Treatments	Sugar (%)			Ascorbic acid (%)		
	Root	Shoot	Leaf	Root	Shoot	Leaf
T <sub>1</sub>	1.90	3.00	2.30	1.20	1.20	1.30
T <sub>2</sub>	2.60	3.37	2.50	1.40	1.40	1.90
T <sub>3</sub>	3.10	4.10	4.50	1.70	1.09	1.25
T <sub>4</sub>	4.10	4.80	4.70	1.80	1.29	1.69
T <sub>5</sub>	5.70	5.60	5.00	1.80	1.41	1.69
T <sub>6</sub>	6.10	6.30	7.30	5.00	1.57	4.05
T <sub>7</sub>	6.50	7.20	7.80	5.03	1.78	5.78
T <sub>8</sub>	8.10	7.20	8.40	10.06	1.99	7.60
T <sub>9</sub>	8.30	10.60	10.60	1.62	1.96	1.99
T <sub>10</sub>	5.60	10.30	9.50	1.34	1.84	2.30
T <sub>11</sub>	5.00	4.50	9.00	1.98	0.56	1.12
T <sub>12</sub>	4.60	4.10	2.10	2.28	1.02	5.15
SEM(±)	0.264	0.346	0.216	0.181	0.100	0.064
CD (5%)	1.279	1.464	1.157	1.059	0.788	0.627
CV	5.872	5.804	4.437	8.427	12.934	4.936
LSD (5%)	0.502	0.574	0.454	0.415	0.309	0.245

SEM: Standard Error Mean's; Critical difference, CV: Covariance, LSD: Least Significant Difference between treatment means

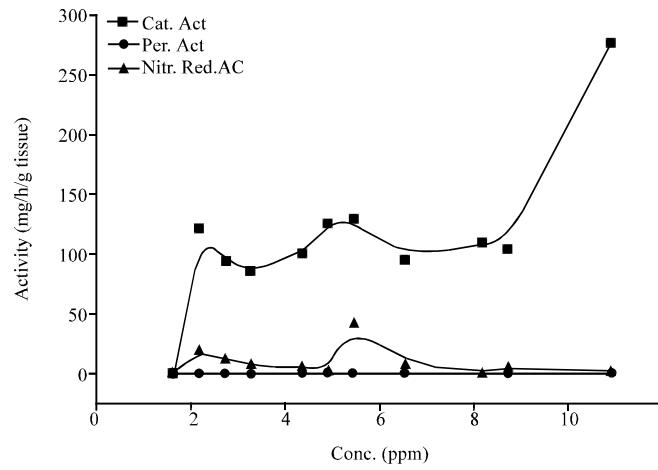


Fig. 1: Changes in the catalase, peroxidase and nitrate reductase activity in leaves of gram seedlings under molybdenum treatment

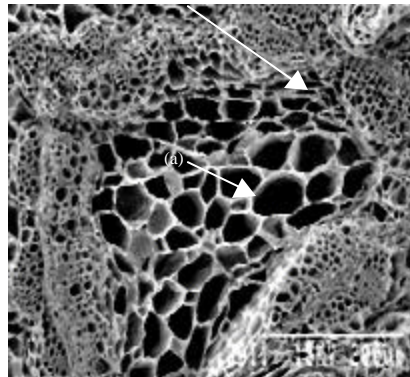


Fig. 2: Control Root (Without Mo Treatment)500x (a)- Alternate arrangement of xylem and phloem

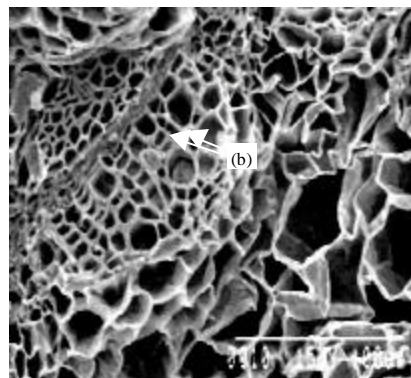


Fig. 3: Control shoot (Without Mo Treatment)500x (b)Prominent cambium layer

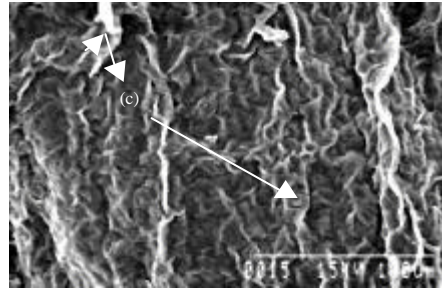


Fig. 4: Control leaf (without Mo treatment) 500x (c)- Distinct stomatal opening

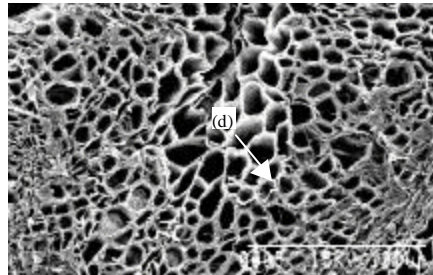


Fig. 5: 1.5 ppm Mo treated Root 500x (d) net like appearance due to Reduction of xylem and phloem vessels

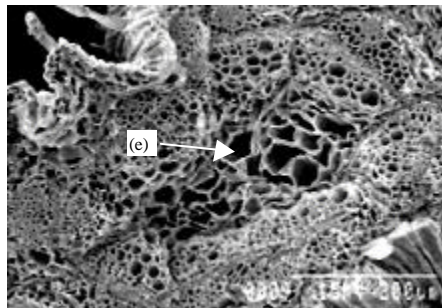


Fig. 6: 1.5 ppm Mo treated Shoot 500x (e) Enlarged metaxylem

There is considerable variation in the anatomical structure of root, shoot and leaves of gram seedlings under various treatment of molybdenum. For root samples under molybdenum treatment of 1.5 ppm the vessel size of xylem and phloem became reduced. There is an aggregation of parenchymatous and sclerenchymatous tissue giving a net like appearance (Fig. 5). At 3 ppm of molybdenum treatment there is complete agglomeration of xylem, phloem, parenchymatous, sclerenchymatous tissue in root rendering the structures undistinguishable visually (Fig. 8). At the highest concentration of molybdenum (10 ppm) under present investigation there is complete distortion of the xylem layer. The xylem vessels became gradually fused giving a hole like appearance along with complete destruction of Phloem and cambium layer (Fig. 12).



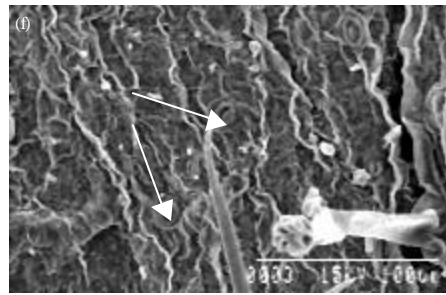


Fig. 7: 1.5 ppm Mo treated leaf 500x (f) Distinct stomatal openings

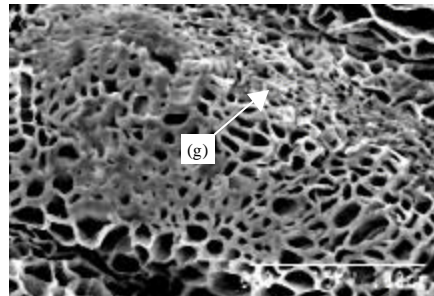


Fig. 8: 3 ppm Mo treated root 500x (g) Complete agglomeration of different tissue layers

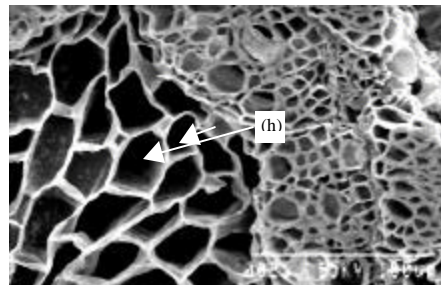


Fig. 9: 3 ppm Mo treated shoot 500x (h) Enlarged vessel of metaxylem

For shoot samples under molybdenum treatment of 1.5 ppm there is considerable increase and decrease in the vessel size of xylem and phloem respectively. Metaxylem was found to be enlarged in size and indistinguishable prototype present (Fig. 6). Less than 3 ppm concentration of molybdenum treatment there is significant increase in xylem. As a result phloem and other parenchymatous tissue cannot be distinguished properly (Fig. 9). There is maximum increase in the diameter of xylem under highest concentration (10 ppm) of molybdenum treatment (Fig. 12).

In the present investigation, leaf samples under various treatment of molybdenum showed significant anatomical variation in the structure. Under 1.5 ppm treatment of molybdenum, distinct stomatal openings were found with respect to control. There are gradual reductions of distinctness

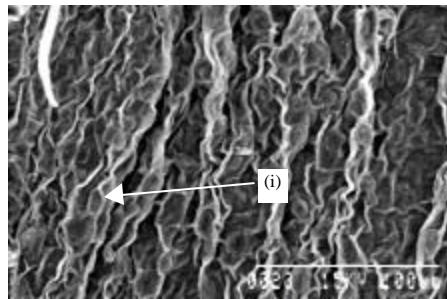


Fig. 10: 3 ppm Mo treated leaf 500x (i) Bulging appearance of stomata

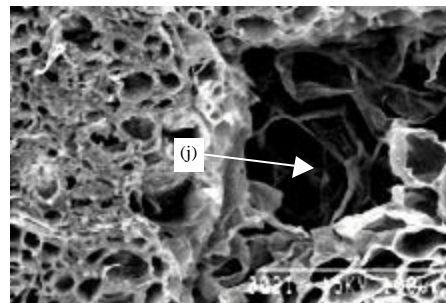


Fig. 11: 10 ppm Mo treated Root 500x (j) Hole like appearance due to Fusion of xylem vessels

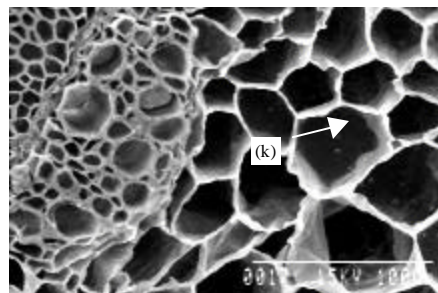


Fig. 12: 10 ppm Mo treated Shoot 500x (k) Enlarged vessel of metaxylem

of mesophyll tissue (Fig. 7). There is complete distortion of mesophyll tissue in leaves under 3 ppm treatment of molybdenum. The stomata were bulging in appearance (Fig. 10). At 10 ppm there is unconsolidated and undifferentiated mass of cells (Fig. 13).

## DISCUSSION

The subsequent increase in root length and shoot length upto treatment  $T_6$  is an indication that molybdenum significantly influenced the growth of gram seedlings as measured in the root length and shoot length. Beyond this treatment there was reduction in both root length and shoot length

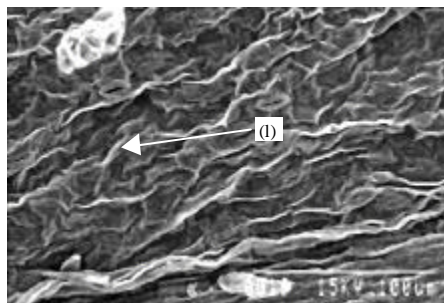


Fig. 13: 10 ppm Mo treated leaf 500 xs (l) Undifferentiated mass of cells

with increased molybdenum concentration thus indicating towards that the optimum molybdenum dose for plant growth might have been reached at  $T_0$  treatment and higher concentration above the optimum dose is inhibitive for plant growth. Present findings were similar with some earlier works of Gungula and Garjila (2006).

In the present investigation, the fresh and dry weight of gram seedlings were reduced with respect to the higher molybdenum treatment which might be due to the produced poisonous effect of this concentration on plants. Present findings corroborates with the earlier findings of Nautiyal *et al.* (2004). In addition, the results indicated that *Cicer arietinum* could be tolerant to the higher molybdenum concentration and there was a large concentration range between molybdenum deficiency and molybdenum poisoning.

The effect of molybdenum in chlorophyll is indirect since when nitrogen is supplied as nitrate, in the absence of molybdenum, plants have shown a poor growth and less chlorophyll (Marschner, 1995). In the present investigation the higher level of chlorophyll a, b and total chlorophyll in leaves of crop plants under molybdenum treatment could be due to a higher amount of N incorporated into the chlorophyll biosynthesis, since nitrogen is a constituent of this molecule. Present works are supported by the earlier findings of Jones (1998) and Raven *et al.* (1999).

Molybdenum has a close relationship with metabolic products of carbon and nitrogen metabolism. In the present investigation higher level of molybdenum treatment may have promoted the content of carbon assimilation products such as total soluble carbohydrates in different parts of the plant body. Present findings corroborates with some earlier findings of Hu *et al.* (1998) and Xuecheng *et al.* (2002).

Ascorbic acid is an antioxidant and in association with other component of the antioxidant system ascorbic acid protects plants against oxidative damage (Streb *et al.*, 2003). In the present investigation, to combat the oxidative stress condition of gram seedlings under higher molybdenum concentration and elevated catalase activity the level of ascorbic acid in different plant parts gradually increased. This may help to scavenge the reactive oxygen species produced under oxidative stress induced by higher concentration of molybdenum.

The leaf nitrate reductase activity of *Cicer arietinum* decreased at the higher concentration of molybdenum which may be due to the ratio change of nitrate nitrogen to ammonia nitrogen in plants (Men and Li, 2005) or high molybdenum concentration changed the corresponding elements involved in synthesizing molybdenic enzymes (Liu, 2002).

Higher rate of catalase activity in leaf of the plants under higher concentration of molybdenum suggests plants under excess treatments of molybdenum will be under oxidative stress leading to

higher production of Reactive Oxygen Species (ROS) (Xuecheng *et al.*, 2006). On the other hand peroxidase activity in leaf was found to be reduced in the present investigation which may be due to its antagonistic nature to catalase. Peroxidase within the plant system is oxidized metabolically to produce hydrogen peroxide. This H<sub>2</sub>O<sub>2</sub> (chemically active) reacts with wide range of macromolecules destroying their functions. Catalase prevents this function by converting H<sub>2</sub>O<sub>2</sub> to oxygen in water (Smirnoff, 1993). Therefore catalase and peroxidase is antagonistic to each other. Thus in the present investigation higher molybdenum concentration decreased peroxidase activity and a subsequent rise in catalase activity.

There is considerable variation in the anatomical structure of root, shoot and leaves of gram seedlings under molybdenum treatment in comparison to control. The gram seedlings without molybdenum treatment (control) showed gross anatomical distribution of different tissue layers. From 1.5 ppm treatment of molybdenum there is gradual distortion of the structure of xylem, phloem parenchymatous and sclerenchymatous tissue layers cell layers of root indicating the deleterious role of molybdenum in plant system in excess amount. The gradual enlargement of vessel size of xylem and phloem may be attributed towards the higher absorption of molybdenum in the tissue layers during conduction of water and solutes in the crop seedlings. At the highest concentration of molybdenum (10 ppm) there is complete distortion of xylem, phloem and cambium layer of roots indicating towards overgrowth of cell layers of the said regions under current investigation (Fig. 11). In leaf samples, upto 1.5 ppm concentration distinct stomatal structure were obtained. Gradual increase in the molybdenum concentration reduced the distinctness of mesophyll tissue and stomatal structure indicating 1.5 ppm concentration of molybdenum as threshold limit for growth and development of gram seedlings under laboratory condition.

## CONCLUSION

The study indicated molybdenum application with different concentrations (1.5 to 10 ppm) promoted higher seed germination, plant growth, fresh weight and dry weight as well as accumulation of total soluble sugar and chlorophyll content in *Cicer arietinum* seedlings. Appropriate amount of molybdenum was helpful to promote molybdoenzyme activity and promote the good growth of anatomical regions of different plant parts. Although, Molybdenum at higher concentration was inhibitive for certain enzyme activity and it also reduced the fresh and dry weight of gram seedlings but the range of molybdenum concentration in this study did not produce poisonous effect on plants. The study also indicates that excessive amount of molybdenum may cause problem towards good plant growth and productivity. Therefore, optimum treatment of molybdenum needs to be employed upon crop plant for sustainable production of crop.

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