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REVIEW ARTICLE

# INFLUENCE OF MICRONUTRIENTS (MOLYBDENUM AND IRON) APPLIED IN COMBINATION WITH *RHIZOBIUM* ON BIOCHEMICAL PARAMETERS OF *VIGNA RADIATA* (L.)

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**ABSTRACT**

A field experiment was carried out to evaluate the effect of different levels of molybdenum & iron on *Vigna radiata* (L.) plants. The experiments were conducted in the field of Botany department of CCS University, Meerut during Kharif season 2017. Different levels of molybdenum (3, 6 and 9ppm) and iron (30,60 and 90 ppm) along with *Rhizobium* were applied. Results revealed that maximum chlorophyll content, nitrogen content, protein content, proline content and legheamoglobin content were observed in those treated plots where Mo 6ppm and Fe 60 ppm (with *Rhizobium)* were applied . Results shows that with increasing the amounts of Mo and Fe up to a certain level in soil, biochemical parameters (nitrogen, protein, proline, chlorophyll and legheamoglobin) significantly increased. Moreover, in the present study when we applied the Mo and Fe above this level (6ppm and 60ppm) the biochemical parameters decreased. The present study suggests that plants require these micronutrients in trace amount and their optimum level is beneficial to improve the legumes growth when applied in combination with *Rhizobium*.

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## INTRODUCTION

Pulses have occupied enormous significance in recent years as an important component of Indian economy. Pulses are important part of vegetarian diet as rich source of protein and full fill the major portion of the protein requirements of animals and human nutrition. Mostly proteins consumed in our country obtained from vegetables (Malik et al, 2015). Pulses are short duration crops (1-4 months) having deep root system, so these are drought tolerant due to their ability to absorb more water. Pulses are also suitable for multiple cropping systems and have an important role in maintaining soil fertility (Malik, 2015). Mung bean (*Vigna radiata* L. Wilczek) is one of the major pulse crops grown in India. Mung bean can supplement the cereal-based diet to improve the nutritional value of food and has a special importance in intensive crop production system of the country for its short growing period (Ahmed *et al*., 1978). Mungbean is capable of fixing atmospheric nitrogen through *Rhizobium* species living in root nodules. Inoculation of mungbean with *Rhizobium* increased plant height, leaf area, photosynthetic rate and dry matter production (Mehboob et al., 2012). The use of BNF (Biological nitrogen fixation) technology in the form of *Rhizobium* inoculants in grain legumes can supplement the expensive fertilizer, particularly for improving the production of food legumes in the country. Mung bean needs some macro-nutrients (N,P,K,Ca++, Mg++,Na, Si, S) and micro nutrients (Mo, Ni, Cu, Mn, Cl, Zn, Cr, Cu etc.) for its normal growth. Some of these micronutrients and macronutrients plays important roles in biological nitrogen fixation and symbiosis between legumes and *Rhizobium,* for instance, molybdenum is a component of the nitrogenase enzyme complex. *Rhizobium* (which fixes nitrogen) needs molybdenum for nitrogen fixation process. Application of molybdenum into the soils which are deficient of this element has increased the contents of potassium, phosphorus and crude protein in *Vigna radiata* L. Molybdenum has positive impacts on growth, yield and nodulation in pulses. Iron (Fe) is very important micronutrient for leguminous crops. Iron deficiency is a common nutritional disorder observed in many legumes crops (Erskine *et al.,* 1993) including *Vigna radiata* L. Losses in the yield of leguminous plants varied between 18 and 25%. Iron is a constituent of the nitrogenase enzyme, leghemoglobin and ferrodoxin. The bacteria have used this element during the nitrogen fixation period. Iron deficiency generally decreases nodule formation, leghemoglobin production and nitrogenase activity, leading to low nitrogen concentrations in the shoots in legumes. Iron and molybdenum treatment have been demonstrated to help the biological nitrogen fixation process, improving the yield and growth of the *Vigna radiata*. It is well known that nitrogen

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fixation can only go actively if the crop is healthy and the nutrient supply adequate (Brar and Sidhu, 1992). Both Mo and Fe are mandatory for plant growth and yield. It is necessary to investigate that how much quantity of these nutrients are required by plant and enhance the growth and yield of *Vigna radiata.* The purpose of this work was to observe the effect of iron and molybdenum on selected biochemical parameters of *Vigna radiata*.

## MATERIALS AND METHODS

The present investigation was organized to find out the "Combined effect of *Rhizobium*, molybdenum and iron on physio-chemical properties of *Vigna radiata* L". The details of material used for experimental purposes and techniques adopted in the present investigation are described as follows

**Geographical Situation:** Meerut is located between $77^0$ 45E longitude and $29^0$ 01N latitude at an altitude of 237 meters above sea level.

**Experimental site:** The University is placed at the distance of 12km from Delhi-Dehradun highway. The aggregate geological territory of Meerut area is 2522 km2. Meerut situated under western plain zone of Uttar Pradesh, sub area of upper Gangetic plain. The research work was conducted during kharif season in 2017-2018 to determine the reaction of *Rhizobium,* Mo and Fe individually and in combination on physiological and biochemical properties of soil and *Vigna radiata* L. The seeds of *Vigna radiata* L. were sown in the field of department of Botany, CCS University, Meerut. The experiment composed in six plots of equivalent size (1×1meter), one plot for the control and remaining five plots for treatments. In the experimental work seeds are inoculated with *Rhizobium* for 12 hours and then sowed in five plots (controls have no *Rhizobium*). Different concentration of Mo and Fe in combination or separately (as given below) mixed with one liter distilled water for each treatment and sprayed uniformly except in control. Treatments are given as follows

1. Control
2. Mo (6ppm)+ *Rhizobium*
3. Mo (6oppm) + *Rhizobium*
4. Mo&Fe (3+30ppm) + *Rhizobium*
5. Mo&Fe(6+60ppm)+ *Rhizobium*
6. Mo&Fe (9+90ppm) + *Rhizobium*

### Material used

- Pure seeds of *Vigna radiata* L. were procured from Indian Agriculture Research Institute, New Delhi.
- *Rhizobium* was acquired from from Indian Agriculture Research Institute, New Delhi.

### Other Details (Experimental Details)

- Total no. of block - 6
- Control - 1
- Total no. of treated plots - 5
- Plot size (area of plot) - 1X1 meter.

### Determination of protein

The protein was estimated by the method adopted by Bradford (1976). The following formula was used for the measurement of protein content

$$\text{Protien}\left(\frac{\text{Mg}}{\text{g}}\right) = \frac{\text{O.D.} \times \text{Factor} \times \text{Dilution (if any)} \times 1000}{100 \times \text{Total volume/volume of replicate}}$$

### Legheamoglobin content

Legheamoglobin quantities of the nodules were measured spectrophotometrically as haemochromogen according to the method of Bergersen (1980). Legheamoglobin content calculated by using the following formula

$$\text{Legheamoglobin Protein} = \frac{\text{LB/g fresh weight of nodule X 100}}{\text{Protein/g/ freshweight of nodules}}$$

### Estimationof chlorophyll content

Arnon's method (1949) is used to estimate the total chlorophyll content of plants**.** Following formula was used for calculation

$$\text{Chl. a (mg/g f } wt.) = \frac{12.7\ (\text{A663}) - 2.69\ (\text{A645}) \times \text{V}}{1000 \times W}$$

$$\text{Chl. b (mg/g f } wt.) = \frac{22.9\ (\text{A645}) - 4.89\ (\text{A663}) \times \text{V}}{1000 \times W}$$

$$\text{Total Chl. (mg/g f } wt.) = \frac{20.2\ (\text{A645}) - 8.02\ (\text{A663}) \times \text{V}}{1000 \times W}$$

Where,

V = final volume of chlorophyll extract
A = absorbance at specific wavelength
W = fresh weight of tissue extract

**Estimation of total nitrogen:** Total nitrogen was estimated by the method as suggested by Snell and Snell, (1967).

**Estimation of total proline:** Total Proline was estimated by the method of Bates *et al.* (1973).

### Statistical analysis

IBM- SPSS was used for stastical analysis (ANNOVA) and for mean plots.

## RESULTS AND DISCUSSION

### Nitrogen Content

Maximum nitrogen content was observed when Mo and Fe (6 ppm & 60 ppm respectively) applied. Minimum amount of nitrogen was observed at control. There were gradual increases in nitrogen content when *Rhizobium* + Mo + Fe were applied. However in treatment 2 and 3 where only Mo and only Fe were applied with *Rhizobium*, nitrogen content was higher as compared to control. These results show positive impacts of Mo and Fe on leguminous crops. Nitrogenase enzyme (Key enzyme of Biological nitrogen fixation) contains two proteins: Mo-Fe protein (molybdenum+ iron + protein) and Fe protein (have iron and protein) Molybdenum is required to the *Rhizobium* bacteria for proper function of nitrogenase enzyme which involved in nitrogen fixation. Molybdenum is the co-factor for enzyme nitrate-reductase also which involved in nitrogen assimilation (Hansch and Mendel, 2009). The application of molybdenum in deficient soil encourages nitrogen fixation and nodule formation (Rahman *et al.* 2008).

Like Mo iron also plays mandatory role in several enzyme systems in which heme functions as the prosthetic group. Heme enzyme systems comprise the catalases, peroxidases and several cytochromes. Therefore, iron and molybdenum are essential components for the nitrogen fixation process). When Fe and Mo are present in optimum amount more nitrogen is fixed through biological nitrogen fixation and its results in high nitrogen availability for plant use (Meagher *et al.* 1991. Significant effect of applied micronutrients on seed nitrogen content was noticed by Campo *et al.* (2009). Similar results were also reported by Khan *et al.* (2014).

**Protein Content**

The beneficial effect of Mo and Fe on yield and yield contributing parameters could be attributed to their vital role in the function of enzymes for biological processes in plants which lead to increase in yield components. It is involved in several enzyme systems and required in biological nitrogen fixation and improves *Rhizobium* efficiency of nitrogen fixation. Fe and Mo have stimulatory effects on protein content. Maximum amount of protein is present at treatment 4 (6 ppm Mo + 60 ppm Fe + *Rhizobium*) in comparison to control where minimum amount of protein content was observed. Molybdenum and Fe have regulatory role in Biological nitrogen fixation and enhance the nitrogen content. Nitrogen is the building block of amino acids, so it has positive impact on protein content. Vieira *et al.* (1998) stated that molybdenum application resulted in enhanced total nitrogen accumulation in seeds as well as protein content of *Phaseolus vulgaris* L. Similar reports were also reported by Togay (2015) which showed that at a optimum level of iron and Mo have positive impact on protein content.

**Proline content**

Proline is a stress indicator amino acid that accumulates under stress conditions and helps to induce tolerance for that stress. In present investigation maximum proline accumulation was observed at control while minimum in plants treated with 6 ppm Mo and 60 ppm Fe with *Rhizobium*. These results are in agreement with those of other investigators using different plants as experimental materials (Chen *et al.* 2001). However there are also slightly increase at 9 ppm Mo and 90 ppm Fe, may be due to the heavy metal stress as these micronutrients are required by plants in traces and their high amount induce heavy metal toxicity. Proline increases the stress tolerance of the plants through osmoregulation, protection of enzymes against denaturation, and the stabilization of protein synthesis. In addition, proline could be involved in metal chelation in the cytoplasm (Farago and Mullen, 1979).

**Legheamoglobin content**

The single most abundant protein that the plant host and symbiotic bacteria *Rhizobium* makes in the nodule is legheamoglobin, an iron containing protein. In the present study we observed positive effect of selected micronutrients (Mo &Fe) with *Rhizobium* inoculation on *Vigna radiata* L. Minimum amount of legheamoglobin was obtained in control and maximum amount of this protein was observed when both Fe and Mo were applied at 60 ppm and 6 ppm, respectively. In the bacteria, nitrogenase and nitrate reductase contain FeS clusters and the former has the co-factor Mo-Fe at the active site for N2 reduction. Further, bacteroids have a very high respiratory demand, requiring abundant cytochromes and other electron donors, each with their own Fe centers.

**Means Plots and Graphs**

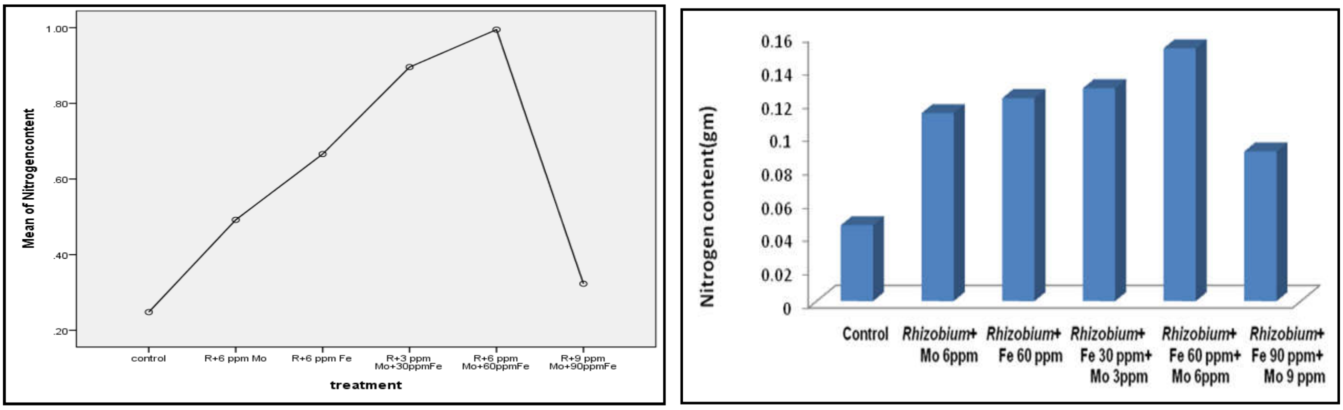


**Figure 1. Nitrogen content of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing**

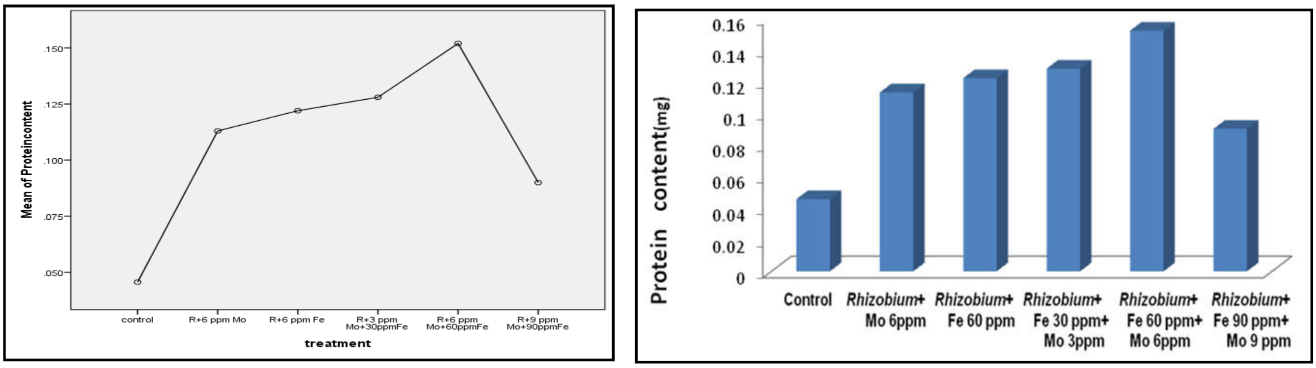


**Figure 2. Protein content of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing**

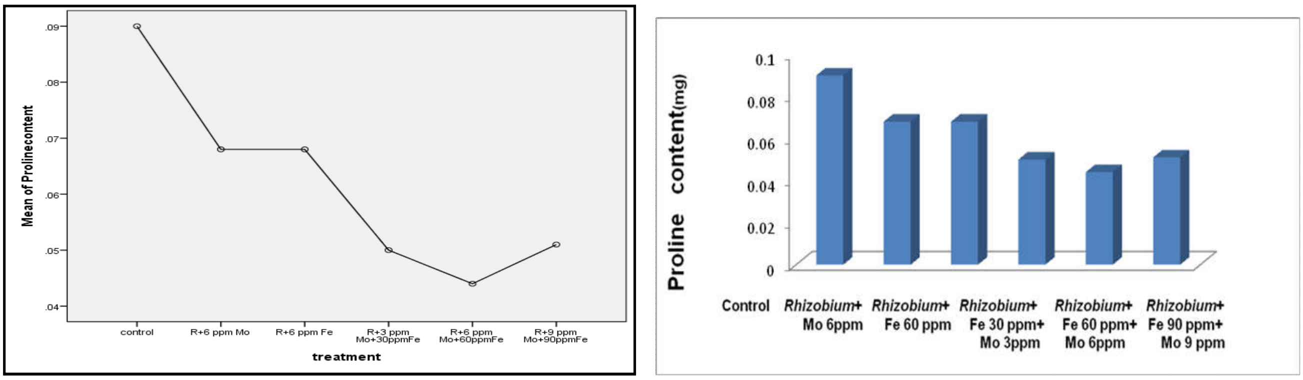


**Figure 3. Proline content of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing**

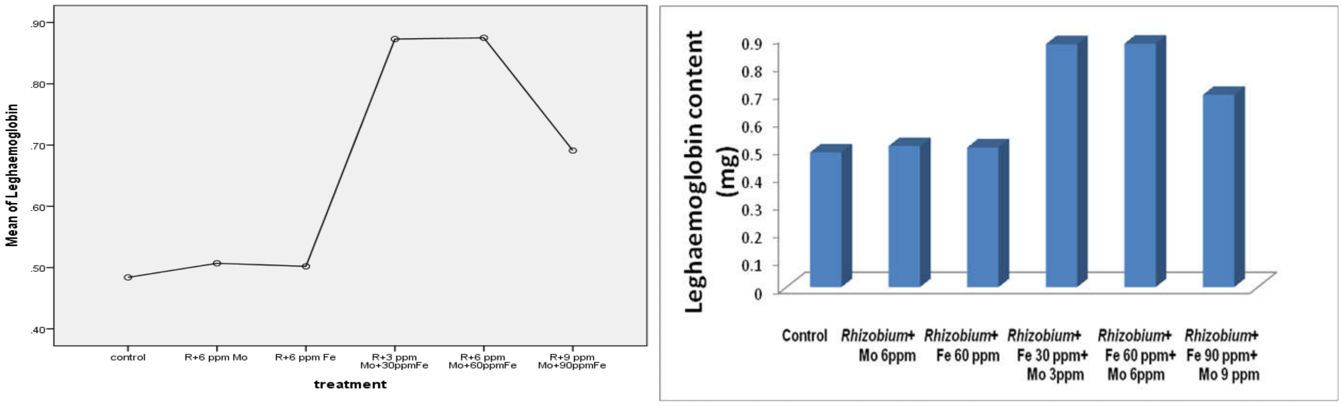


**Figure 4. Legheamoglobin content of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing**

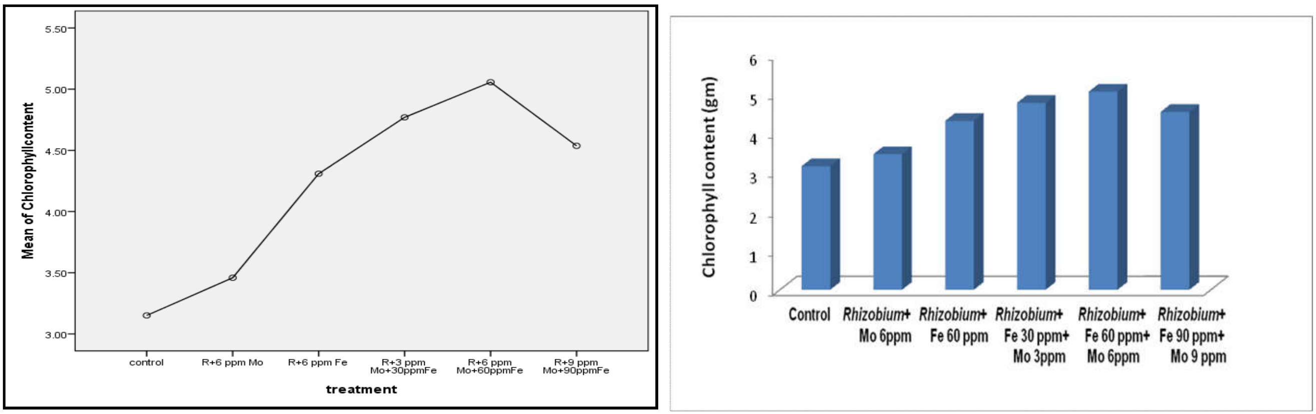


**Figure 5. Chlorophyll content of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing**

Therefore, in legumes iron is required in a greater amount for nodule formation than for host plant growth. Its relevance to N2 fixation is clear, given that the Mo in 'Fe-Mo' cofactor is the important part of the nitrogen reduction process. An interrelation amongst molybdenum and iron has been determined by Berry and Reisenauer (1967), who observed that molybdenum supply significantly increases the capacity of tomato plants to absorb Fe++. Molybdenum is important factor of nitrogen fixation and affects the legheamoglobin content significantly. Significant effect of Mo and Fe on legheamoglobin (Lb) content can be attributed to high amount of Lb as these micronutrients are the key components of nitrogenase enzyme and nitrate reductase that enhances bacterial growth and symbiotic efficiency (Solaiman, 1999).

## Chlorophyll content

Lowest chlorophyll content recorded in control and highest chlorophyll content was observed when 6 ppm Mo and 60 ppm Fe was applied. Iron is an important micronutrient for legumes. It plays an important role in synthesis and maintenance of chlorophyll in plant. It is absorbed by plants as the ferrous ion ($Fe^{+2}$),which is necessary for the formation of chlorophyll and functions in some of the enzymes of the plant's respiratory system (Schneider *et al.*, 1968) Iron deficiency in soybean results in chlorosis (Rotaru and Sinclair, 2009). Similarly molybdenum (Mo) plays an important key role in chlorophyll synthesis, it is absorbed as MoO4. Molybdenum deficient plants exhibit poor growth and low chlorophyll content (Gupta and Lipsett, 1981; Gupta *et al*., 1991; Marschner, 1995). Molybdenum deficient plants exhibit poor growth and low contents of chlorophyll and ascorbic acids and shows reduced leaf blade formation, inter-veinal mottling and chlorosis around edges and tips of older leafs (Marschner, 1995; Liu, 2002).So Mo and Fe have important role in chlorophyll synthesis and significantly increase the amount of chlorophyll. The results have hormony with Malik *et al*. (2015), they observed the effect of Molybdenum on chlorophyll content which show positive response of molybdenum on plant chlorophyll content. Similar results were observed by Nazirkar (2015) when they applied molybdenum, zinc and iron on soyabean plants. They find higher chlorophyll content at 40 DAS (28.06 mg 100 g-1) of soybean was observed under foliar application of zinc and iron with Molybdenum. Lehaemoglobin binds to oxygen and provide suitable atmosphere to *Rhizobium* bacteria for nitrogen fixation. A particular high iron requirement exists in legumes for the heme component of legheamoglobin.

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