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VIGNA RADIATA (L.) YIELD AND ITS QUALITY INFLUENCED BY *RHIZOBIUM* INOCULATION AND DIFFERENT CONCENTRATION OF IRON AND MOLYBDENUM.

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Abstract: The aim of this research was to study the effect of different concentrations of application of molybdenum and iron in combination with *Rhizobium* on the vegetative growth and yield of *Vigna radiata* (L.) under field conditions. Different concentration of Mo (3,6 and 9 ppm) were given to plants whilst Fe was given at 30,60 and 90 ppm. Results showed that 6 ppm Mo and 60 ppm Fe significantly improved the growth characters, yield and the nodulation efficiency of *Vigna radiata* (L.). However when the amount of these micronutrients increase (9ppm Mo and 90 ppm Fe) there are decrease in growth and yield parameters of the plant. From these results, it could be recommended that molybdenum and iron individually or in combination with *Rhizobium* are important and essential elements in the chemical fertilization management system for the legume production and can be used to improve the legumes yield and production. It can be concluded that these micronutrients are vital for optimum activity of *Rhizobium* and plants inoculated with *Rhizobium,* enhanced mung bean productivity.

Keywords: *Vigna radiata* (L.)*, Rhizobium,* Molybdenum, Iron, Nodulation.

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INTRODUCTION

Around 60–70% of Indian population (directly or indirectly) depends upon agriculture. It fulfills the basic need of human beings and animals. It is an important source of raw material for many agro-based industries. In India varieties of crops are domestically grown because of diverse environmental conditions which make India a Biodiversity-rich country. It includes rice, wheat, jawar, bajara, maize, barley, cotton, jute, potato, cauliflower, banana, legumes, etc.

Even "nutritionally balanced food" as defined over 1000 years ago consisted of pulses, besides cereals, vegetables and fruits, and milk products (Ayachit, 2002). Today, nutritionists tell us that pulses are important because they provide the essential proteins. Pulses, the food legumes, have been grown by farmers since millennia, and these have contributed in providing nutritionally balanced food to the people of India (Nene, 2006).Legumes which are commonly grown in india are Pea, black gram, common bean, pigeonpea, chickpea, soyabean, mung bean, etc.

Mungbean (*Vigna radiata* L. Wilczek) or green gram is an important legume crop. It is a great source of proteins, vitamins and minerals, particularly in South Asia. It is a self-pollinated crop having 22 chromosomes with a genome size of 579 Mb/1C.It has high nutritive value and has advantage over the other pulses (Kaur *et al.*, 2017**)**. The seed contains 24.20% protein content, 1.30% fat and 60.4% carbohydrates; calcium (Ca) is 118 and phosphorus (P) is 340 mg per 100 g of seed, respectively (Imran *et al.*, 2015). Its capacity to restore soil fertility through nitrogen fixation makes it a valuable crop.

Nitrogen fixation is the process, which as a component of the biogeochemical nitrogen cycle, drives the conversion of atmospheric N2 into ammonium ions. The major source of fixed-N to the biosphere is biological N2-fixation by microorganisms collectively known as diazotrophs; they provide about 60% of the total annual input. In leguminous and few some non-leguminous plants, nitrogen fixing bacteria live in small outgrowths called nodules. The plant roots supply essential minerals and newly synthesized substances to the bacteria (Burdass and Dariel, 2002). Within these nodules, the bacteria do nitrogen fixation and the plant absorbs the ammonia. The soluble form of nitrite and nitrate can be assimilated by plant's roots and utilized in synthesizing proteins and nucleic acids (Rajeswari *et al.*, 2017).

Mungbean is naturally nodulating crop to some extent and the degree of nodulation varies with the area and culture being used. Several key abiotic factors limit legume productivity and nitrogen fixation in world agriculture. These may be summarized as extremes of temperature, water availability, nutrient availability and toxic soil factors such as salinity and pH. For healthy and vigorous growth, plants need to take up from the soil relatively large amounts of some inorganic elements (macronutrients) and small quantities of other elements known as micronutrients or trace elements (Weisany *et al.*, 2013).

Mineral nutrients have major roles in nitrogen fixation process because these nutrients may influence N2 fixation in pulses at different levels of the symbiotic relationship i.e. infection and nodule formation, hemoglobin formation, and host plant growth (O'Hara, 2001). Robson (1978) summarized the nature of the interaction between nutrient supply and combined nitrogen on legume growth as a means for estimating symbiotic sensitivity to their supply or concentration. Although there is currently experimental evidence for specific requirements for 11 nutrients (B, Ca, Co, Cu, Fe, K, Mo, Ni, P, Se and Zn) for symbiotic development in some species of legume, only four of these elements(Ca, P, Fe and Mo) appear to cause significant limitations on the productivity of symbiotic legumes in some agricultural soils. Fe and Mo are required in high amounts for symbiotic nitrogen fixation for host-plant growth than Cu, Ca and P. (O'Hara, 2001).

Molybdenum is utilized by selected enzymes to carry out redox reactions. Enzymes that require molybdenum for activity include nitrogenase, nitrate reductase, aldehyde oxidase, sulfite oxidase and xanthine dehydrogenase. Nitrogenase and nitrate reductase are the key enzymes of biological nitrogen fixation. Molybdenum is the cofactor for the enzyme nitrate reductase which involved in nitrogen assimilation (Hanschand Mendel, 2009). The application of molybdenum in deficient soil encourages nitrogen fixation and nodule formation (Rahman *et al.*, 2008).

Molybdenum is required by the *Rhizobium* bacteria for proper function of nitrogenase enzyme. As a component of meta-protein nitrogenase which helps in biological nitrogen fixation process and acts as important component which needed for metabolism of nitrogen fixing bacteria. The nitrogenase requires molybdenum element in the process of its metabolism, which acts as electron carrier between oxidized and reduction stages. Molybdenum concentrations in legume nodules can be ten times higher than in leaves. It also acts in enzymes, which bring about oxidation reduction reaction, especially the reduction of nitrate to ammonia prior to amino acids and protein synthesis in the cells of plant.

Like Mo, iron is also required for several enzymes of the nitrogenase complex as well as for the electron carrier ferredoxin and for some hydrogenases. High iron requirement exists in leguminous plants for the heme (iron containing) component of leghemoglobin. Therefore, in legumes iron is required in a greater amount for nodule formation than for host plant growth. Iron deficiency is a common nutritional disorder observed in many crops (Erskine *et al.,* 1993). As Iron is an important component of the nitrogenase enzyme, ferrodoxin and leghemoglobin protein, the bacteria have used iron during the nitrogen fixation period. Deficiency of iron decreases nodule formation, leghemoglobin production and nitrogenase activity, leading to low nitrogen concentrations in the shoots in leguminous plants. Fertilization of iron and molybdenum have been proved to enhance the nitrogen fixation processes, improving growth, yield and nitrogen status of plants. Mo and Fe have positive impact on plant growth when applied individually but their combined effect on legume growth is not well known. So as important component of nitrogen fixation mo and Fe can help to improve the Yield of *Vigna radiata*. Present investigation unveiled the response of *Vigna radiata* when different concentration of Mo and Fe with *Rhizobium* inoculation was applied.

MATERIAL AND METHODS

The present investigation was organized to find out the "Combined effect of *Rhizobium*, molybdenumand 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 district is situated between 29^0 01N latitude and 77^0 45E longitude 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 is 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 Botany department, 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 than 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:

1. Pure seeds of *Vigna radiata* L. were procured from IARI, New Delhi.
2. *Rhizobium* was obtained from IARI, New Delhi.

Other Details (Experimental Details);

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

Germination percentage

Fifty healthy seeds of *Vigna radiata* L. were sown in every plot. All plots were irrigated with tap water. The seed germination percentage was calculated after counting the difference between germinated (coming out of soil) and non-germinated seeds (remaining inside, non emergent). The formula to calculate the seed germination % as given below-

Germination percentage = **Seeds germinated/total seeds x 100**

Nodulation

Plants from six plots for each treatment were uprooted 30 days after seeding (DAS) and were observed for the extent of nodulation parameters such as nodules no., nodules weight(fresh & dry) and nodules volume.

Growth parameters

Growth parameters such as plant height(Root and shoot), Plant weight (fresh and dry) was observed to study the effect of molybdenum and iron on plants physical traits. Five plants were removed from each plot and then measured for experiment.

Yield parameters

Plants of different plots were observed for different yield parameter which was no. of pods per plant, no. of seeds per pod**.**

RESULTS AND DISCUSSION

Seed Germination-

Seed inoculation with molybdenum as well as *Rhizobium* exhibited superior performance over untreated seeds (Table1). The combined seeds treatment with molybdenum and Fe with *Rhizobium* emerged as best and recorded the maximum germination. Due to different levels of iron concentration ranging from complete deficiency to toxicity, increased or decreased the growth rate and physiological components as also reported by Nenova (2006). Results also showed that the emergence rate in molybdenum and iron plots were comparatively better than those plots, which received no molybdenum and iron fertilizer as shown in figure 1. The heavy metals have induced delayed response in case of germination because the number of seeds germinated after 5 days was less in comparison to those which were noticed after 10 and 15 days (Dhankhar *et al*. 2011). However there are reduction in germination at 9 ppm Mo and 90 ppm Fe level. Reduction in seed germination rate can also be attributed to the alterations in the selection permeability properties of the cell membrane. The delay in seed germination in the presence of heavy metal is probably connected with the process of cell-stretching, break and fission alike to the finding and reports of Kumar *et al.* (1995).

Plant Growth –

Molybdenum and iron had positive impact on plant growth. Plant height increased with increasing the amount of Fe and Mo. Total plant height (root+shoot) ranged from 38.6 to 45.5. Lowest plant height 38.6 cm was observed with control and largest plant height was observed in treatment 4 (6 ppm Mo and 60 ppm Fe + *Rhizobium*). Plant height increases with increasing the amount of micronutrients (Fe & Mo) with *Rhizobium* inoculation up to 6 ppm and 60 ppm of Mo and Fe. However, after reaching optimum level of these micronutrients, plant height slightly decrease at 9 ppm and 90 ppm level of Mo and Fe respectively. Similar results are reported by Bhuiyan, *et al.*(2008) who reported that the phosphorus and molybdenum application at the rate of 40 kg P ha^{-1} and 1.0 kg Mo ha^{-1} progressively and significantly increased all growth parameters in mungbean plants. Zahoor *et al*.(2013) observed positive impacts on plant growth in their study on *Glycine max* when they applied Fe and Mo in combination.

Plant Weight-

Results showed significant differences in plant height among various molybdenum and iron treatments for shoot biomass of *Vigna radiata* L.(Table3). However maximum plant weight (Fresh/Dry) was observed at treatment 4 (6 ppm Mo + 60 ppm Fe + *Rhizobium*). In present investigation there were minimum weight was in control. The present findings suggest that a normal level of Mo and Fe produce maximum shoot and root biomass as compared to above and below this level. Caliskan *et al*. (2008) observed a significant increase in shoot and root dry weight in mungbean with Mo and Fe application. These micronutrients (Mo & Fe) improved photosynthetic activity contribute for better plant dry biomass. Such results through have also been recorded earlier by Sarivestava *et al.*(1995).

Pods numbers per plant-

Rhizobium inoculation alone and in combination with micronutrients had a significant effect on the number of pods plant-1. No. of pods were found high when micronutrients (Fe and Mo) were applied with *Rhizobium*as compared to control. There were gradual increases in pod no. per plant, when plants treated simultaneously with both Mo and Fe. This concludes that these micronutrients have positive impact on plant yield. As we know that Mo and Fe work as co-factor in biological nitrogen fixation leading to high nitrogen and high protein content. These parameters directly influenced the yield attributes (no. of pods) of plants. These results show harmony with Ahmad *et al*. (2013).

Seeds numbers per plant-

Results revealed that seeds no. per pod were found high (39) when 6ppm Mo and 60 ppm Fe with *Rhizobium* was given. Control has minimum no. of seeds (23) per pod , these results showed that these micronutrients have positive impact on plant yield. Similar results were obtained by Ahmad *et al*. (2013). However, there is slightly reduction in seed no. at treatment 5 where 90 ppm Fe and 9 ppm Mo were applied with *Rhizobium*. The reason may be attributed to the heavy metal toxicity because these nutrients are required in trace amount and their high amounts have negative impacts on plant growth and yield.

Nodules numbers and volume

Regarding number of nodules per plant its maximum number (13) was observed with 3 ppm Mo +30 ppm Fe with *Rhizobium*. Data showed the lesser number (8) of nodules in control plants. Iron and molybdenum had positive impact on nodulation of plants and all treatments had more no. of nodules as compare to control. But it is not the same record with all nodulation parameters because of occurrence nodules variation in their size and volume. These parameters with maximum impact were noted at 6 ppm Mo and 60 ppm Fe. Molybdenum had a notable influence on N-fixation and metabolism in N2 fixing legumes (Vieira *et al*. 1998). In nodulated legumes, Mo is necessary for the reduction of atmospheric nitrogen (N2) to ammonia by nitrogenase enzyme. It has been established that the symbiotic bacteria require more Mo for N2 fixation than the host plants (O'hara *et al*. 1988). Molybdenum is also essential nutrient for nitrate-reductase and nitrogenase enzyme activity (Westermann, 2005). The symbiotic bacterial enzyme nitrogenase is comprised of Mo-Fe protein which is directly involved in the reduction of N2 to NH3 (Lambers *et al*. 1998) during nitrogen fixation process. Further, application of Fe enhances number of nodules per plant as Fe is an important element for rhizobial nutrition, nodulation, nodule activity and biological nitrogen fixation (O,Hara, 2001). Supply of Mo to bacteroids is therefore, an important process and most likely a key regulatory component in the maintenance of nitrogen fixation in legumes that may influence plant growth (Kaiser *et al*. 2005).However, we observed reduction in nodulation with an increase in the amount of Mo and Fe (9 ppm and 90 ppm). This is attributed to the fact that these micronutrients are required in minute amount by the plants and their high amount can reduce the efficiency of nodulation (Malik *et al*.,2013).

Nodules weight-

Nodules weight (fresh+ Dry) was found high when *Rhizobium*+6ppm Mo + 60 ppm Fe was applied as compare to control. Minimum nodules weights were observed in control. Nodulation is depends upon the symbiotic efficiency of *Rhizobium.* As given above Mo and Fe are the key components of Nitrogen fixation process as cofactor of nitrogenase and nitrate reductase enzyme. These micronutrients have positive impacts on nodules weight and increased the biomass of nodules as well as plants when applied with *Rhizobium*. The results have harmony with (Malik *et al*.,2013). However there is reduction in the nodules weight when 9 ppm Mo and 90 ppm Fe were applied this can be due to the negative impact of metal toxicity because these micronutrients are required in trace amount and their high amount can disturb the legume-*Rhizobium* symbiosis as well as Nodules weight.

Tables

Table 1: Seed Germination percentage of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum

Treatments	5 DAY	10 DAY	15 DAY
Control	62	78	89
***Rhizobium*+6 ppm Mo**	64	82	90
***Rhizobium*+60 ppm fe**	66	86	92
Rhizobium+3 ppm Mo+30ppmFe	70	80	94
***Rhizobium*+6ppm Mo + 60 ppm Fe**	78	94	98
***Rhizobium*+9ppmMo+90ppmFe**	58	70	92

Table 2: Root length and shoot length of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing.

Treatments	Root length	Shoot length	Total length
Control	11.6	27	38.6
***Rhizobium*+6 ppm Mo**	12.4	28.41	40.8
***Rhizobium*+60 ppm fe**	12.4	28.91	41.9
***Rhizobium*+3 ppm Mo+30ppmFe**	13.5	30.1	43.6
***Rhizobium*+6ppm Mo + 60 ppm Fe**	12.8	32.7	45.5
***Rhizobium*+9ppmMo+90ppmFe**	12	28.2	40.2

Table 3: Root and shoot fresh and dry weight of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing

Treatments	Dry wt of plant(g)	Fresh wt of plant(g)
Control	2.516	7.614
***Rhizobium*+6 ppm Mo**	2.764	8.273
***Rhizobium*+60 ppm fe**	2.110	7.708
***Rhizobium*+3 ppm Mo+30ppmFe**	2.350	8.946
***Rhizobium*+6ppm Mo + 60 ppm Fe**	4.637	14.373
***Rhizobium*+9ppmMo+90ppmFe**	2.968	8.599

Table 4: No.of pods and No. of seeds of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing

Treatments	No.of pods	No. of seeds
Control	4	23
***Rhizobium*+6 ppm Mo**	5	26
***Rhizobium*+60 ppm fe**	5	25
***Rhizobium*+3 ppm Mo+30ppmFe**	6	28
***Rhizobium*+6ppm Mo + 60 ppm Fe**	7	39
***Rhizobium*+9ppmMo+90ppmFe**	7	32

Table 5: Fresh and dry weight of nodules of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing.

Treatments	Fresh wt of nodules	Dry wt of nodules
Control	0.095	0.029
***Rhizobium*+6 ppm Mo**	0.124	0.032
***Rhizobium*+60 ppm fe**	0.130	0.034
***Rhizobium*+3 ppm Mo+30ppmFe**	0.137	0.037
Rhizobium+6ppm Mo + 60 ppm Fe	0.227	0.040
Rhizobium+9ppmMo+90ppmFe	0.114	0.033

Table 6: Nodules No. and nodules volume of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing.

Treatments	No. of nodules	Volume of nodules (ml)
Control	8	.12
***Rhizobium*+6 ppm Mo**	10	.80
***Rhizobium*+60 ppm fe**	11	0.69
***Rhizobium*+3 ppm Mo+30ppmFe**	13	1.3
***Rhizobium*+6ppm Mo + 60 ppm Fe**	12	1.9
***Rhizobium*+9ppmMo+90ppmFe**	12	1.3

Graphs-

Figure- 1: Seed Germination percentage of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum.

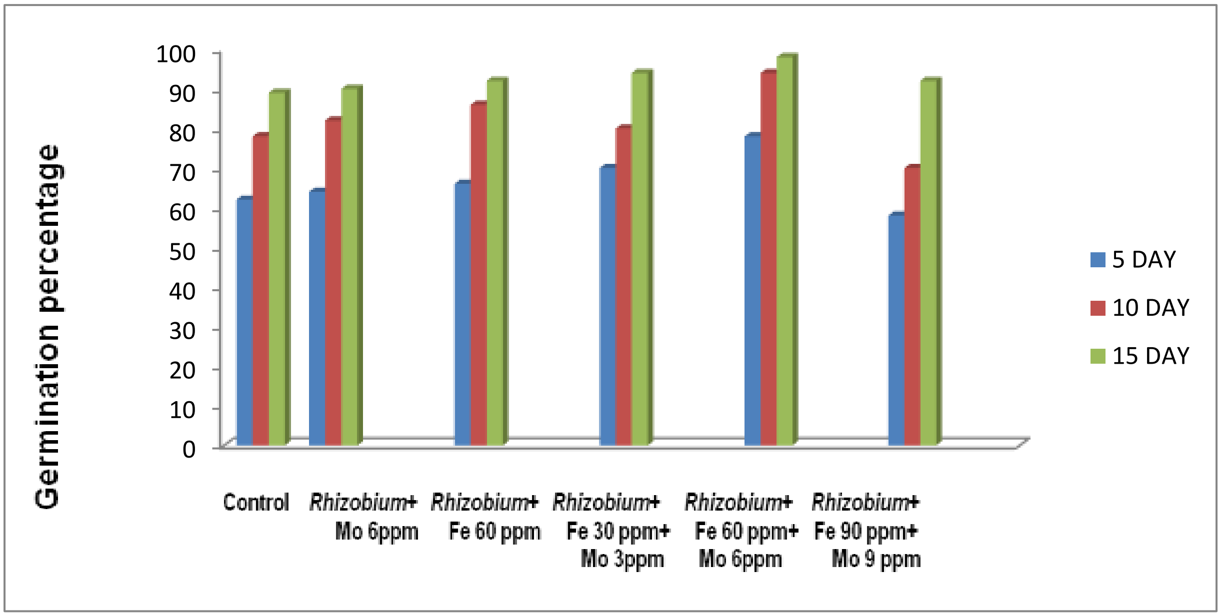


Figure- 2: Root length and shoot length of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing.

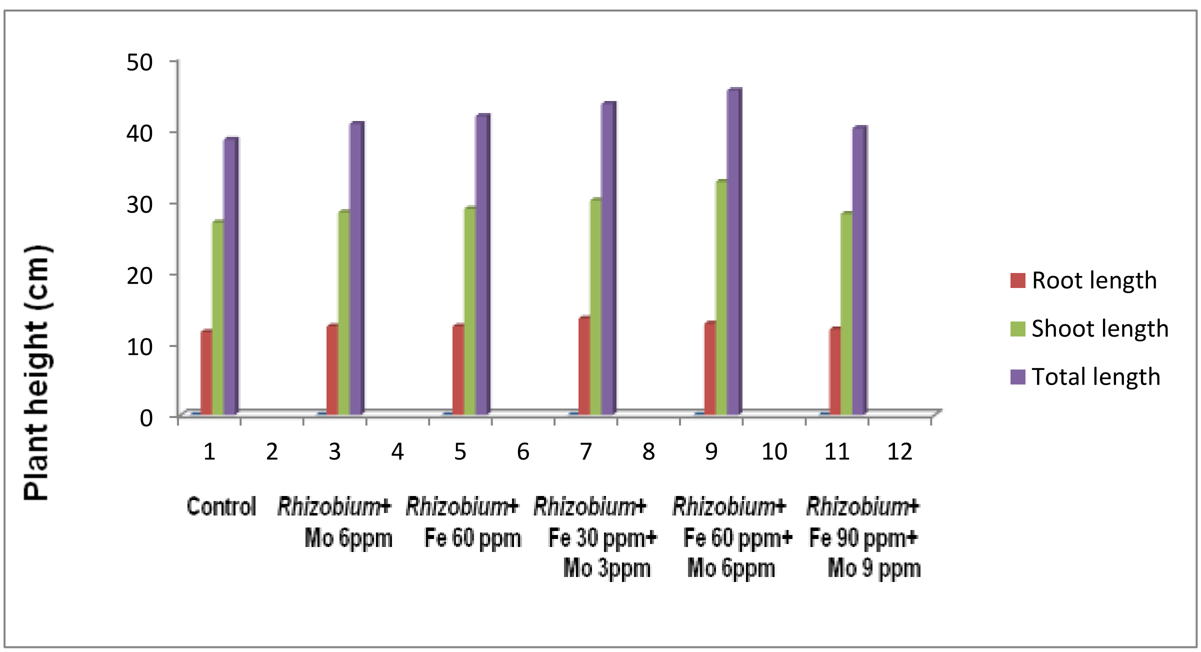


Figure- 3: Root and shoot fresh and dry weight of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing

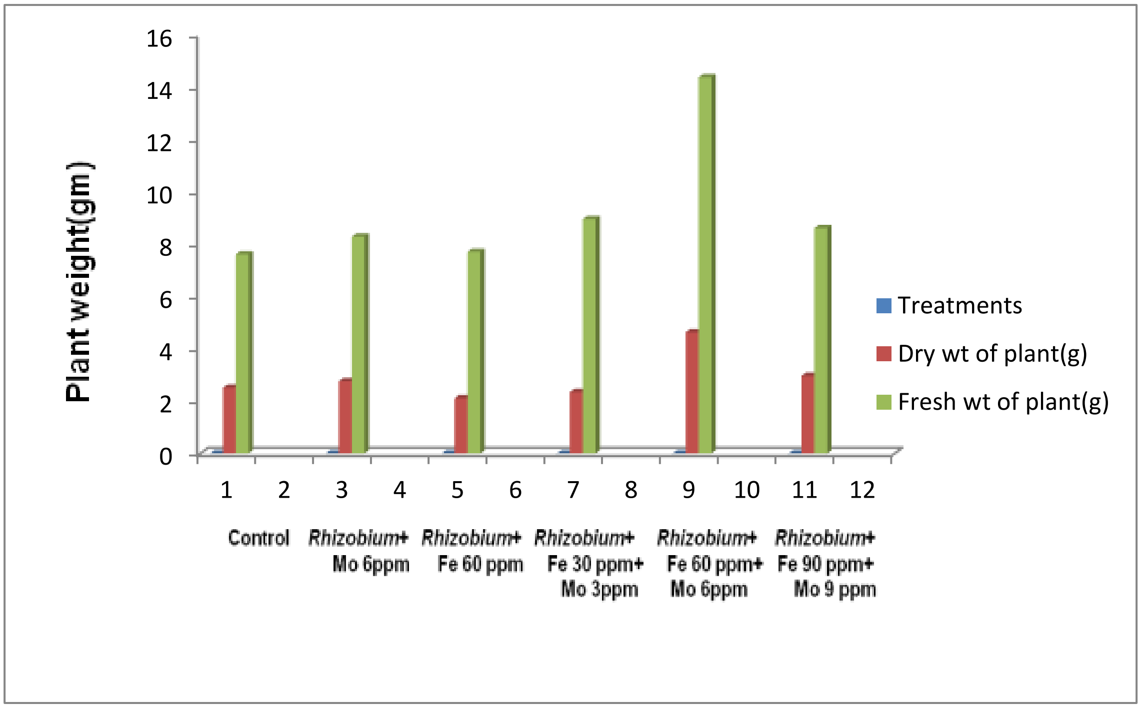


Figure- 4: No.of pods and No. of seeds of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing

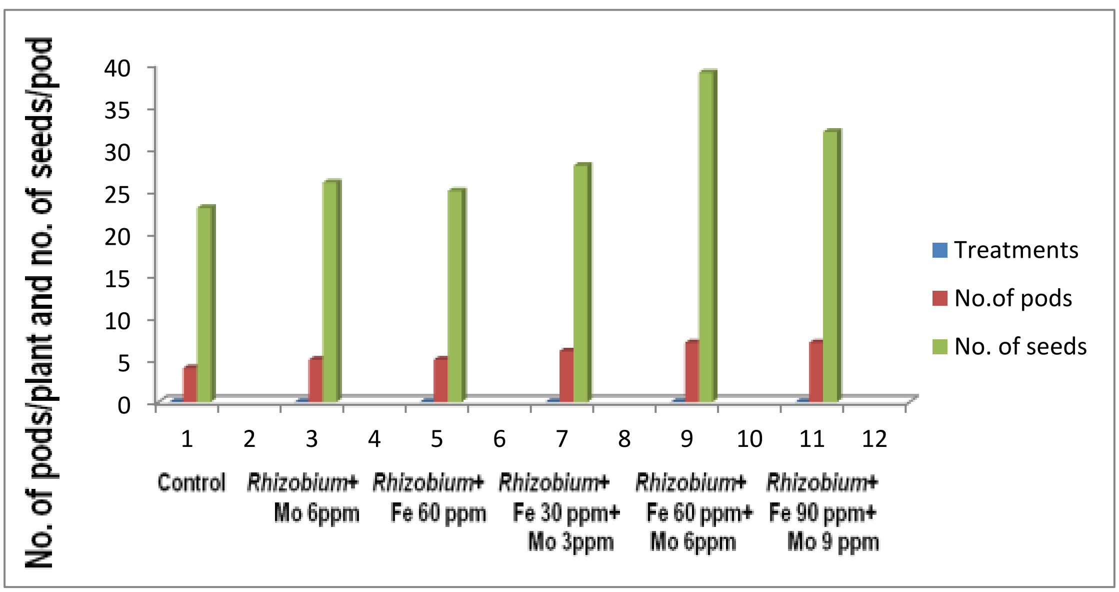


Figure- 5: Fresh and dry weight of nodules of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing.

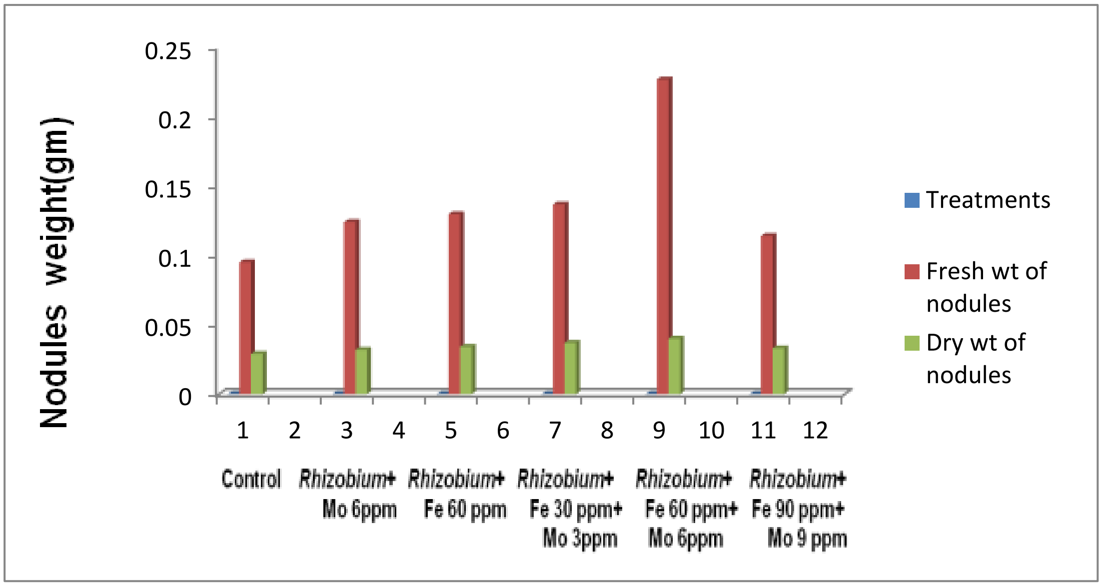
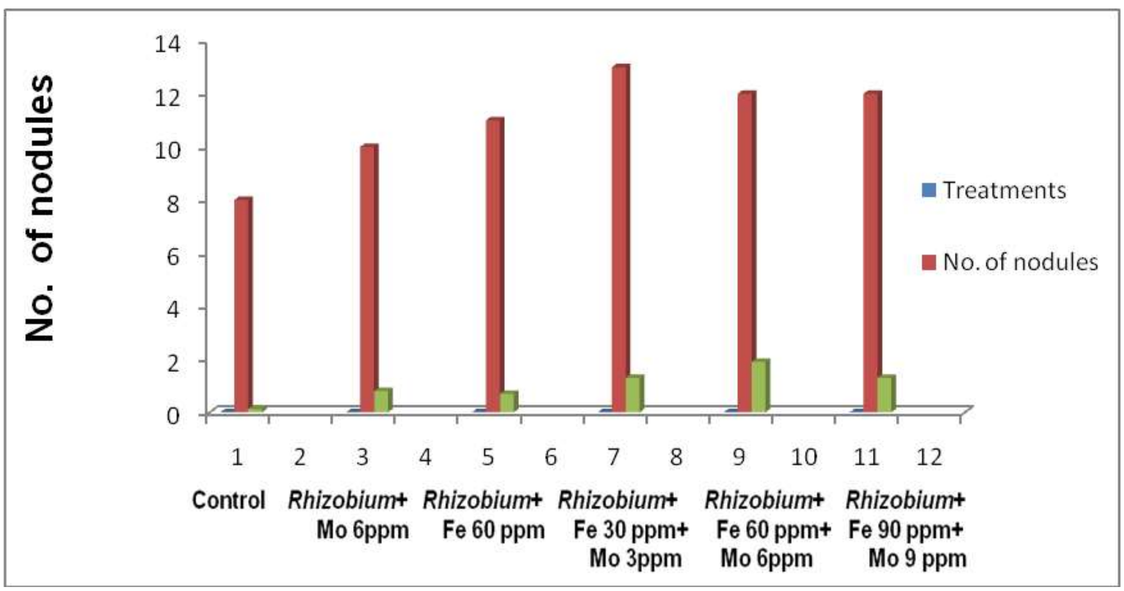


Figure 6: Nodules No. and nodules volume of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of iron and Molybdenum after 30 days of sowing.



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