



Effect of Molybdenum and Zinc on Nodulation, Leghaemoglobin Content and Pod Formation of Horse Gram

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Abstract: An experiment was conducted during kharif season 2013-15 to study the effect of *Rhizobium* inoculation under various concentrations of molybdenum (Mo) and zinc (Zn). The treatment comprising of five levels of Mo and Zn (100, 250, 500, 750 and 1000 $\mu\text{g g}^{-1}$) were evaluated in randomized block design with three replications with and without inoculation with different *Rhizobium* strains (HGR-4, 6, 13 and 25). Application of Mo up to 250 $\mu\text{g g}^{-1}$ increased number of pods with prior inoculation of the strain HGR-4. At this concentration the number of nodules formed were more on the plants inoculated with HGR-4 and HGR-6. The amount of leghaemoglobin was maximum at 250 $\mu\text{g g}^{-1}$ with the strains HGR-4, 6 and 25. Zn enhanced formation of pods up to 500 $\mu\text{g g}^{-1}$ with the strain HGR-25. The number of nodules increased with increase in Zn concentration up to 1000 $\mu\text{g g}^{-1}$ when the plants were inoculated with HGR-13 while leghaemoglobin increased up to 750 $\mu\text{g g}^{-1}$. This study demonstrated that the Horse gram plants inoculated with *Rhizobium* strains HGR-4, 6, 13 and 25 besides having nitrogen fixing ability also have the ability to grow in high Mo and Zn soils.

Key words: Molybdenum, nitrogen fixation, rhizobia, symbiotic efficiency, zinc.

Horse gram [*Macrotyloma uniflorum* (Lam.) Verdc. = *Dolichos biflorus* (Linn.)] is an important pulse crop and is extensively cultivated on light red and gravel soils of Peninsular India. It derives its significance from its adaptability to poor and adverse climatic conditions, which are unsuitable for other pulse crops. In India, Horse gram is cultivated in 1.1 million hectares during both kharif and rabi in the states of Tamil Nadu, Karnataka, Andhra Pradesh and Orissa of South India. It is widely cultivated as a grain legume and fodder crop. Being a legume, it also fixes atmospheric nitrogen and improves soil fertility.

Micronutrients play an important role in increasing legume yield through their effect on the plant, nitrogen fixing process and effective use of major and minor nutrients. Among micronutrients cobalt (Co), boron (Bo), molybdenum (Mo) and zinc (Zn) are essential for the growth of *Rhizobium* and nitrogen fixation. Mo is required for the growth of most of biological organisms including plants, animals (Shanti *et al.*, 2008) and bacteria (Williams and Fraustoda Silva, 2002). Mo is the key element

to nitrogen fixation by legumes (Meagher *et al.*, 1991). It is an essential component of nitrate reductase and nitroge nase, which regulates the reduction of inorganic nitrate and helps in fixing N_2 to NH_3 . Zn is a micronutrient required in small amounts by crop plants, but its importance in crop production has increased in recent years. It is an important micronutrient for nodulation and nitrogen fixation (Smith, 1982) and involved in leghaemoglobin synthesis. Deficiency of Zn in legumes is reported to reduce the number and size of nodules as it is involved in leghaemoglobin synthesis (Marsh and Waters, 1985). There are very few studies on Mo (Dura isamy and Mani, 2001) and Zn effect on symbiotic efficiency and pod yield of horse gram when they were inoculated with rhizobia. Hence, the present work was initiated with the objective to understand the interaction of different native strains of rhizobia under varying Mo and Zn levels.

Materials and Methods

Root nodules were collected from the plants, grown in soil samples collected from different regions of united Andhra Pradesh, India. Using yeast extract mannitol (YEM)

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agar medium containing 0.0025% Congo red dye bacterial isolations were carried out. Furthermore, all the isolates were subjected to authentication test before performing the experiments. The pots filled with soil samples were sterilized in autoclave at 121°C for 3 h each on three successive days to kill all the spores. Seeds of horse gram were obtained from local fields of Andhra Pradesh, India to be used as the experimental material. They were surface sterilized with 70% ethanol for 3 min, sodium hypochlorite for 3 min and were rinsed six times with sterile water and dried. The rhizobial suspension of isolate was grown in YEM broth in flasks shaken at 120 rpm at 28±2°C for 3 days to a cell density of 6×10⁹ cells ml⁻¹. The sterilized seeds were coated with the four rhizobial strains HGR-4 (GQ483457), HGR-6 (GQ483458), HGR-13 (GQ483459) and HGR-25 (GQ483460) by soaking the seeds in liquid culture medium for 2 h using 10% (wt/vol) gum Arabic as adhesive to deliver approximately 10⁹ cells seed⁻¹. The non-coated sterilized seeds that served as control (uninoculated) were soaked in sterilized water only. The uninoculated and inoculated seeds (20 seeds pot⁻¹) were sown in clay pots filled with 2 kg sterilized and unsterilized natural soil and treated with Mo and Zn (100, 250, 500, 750 and 1000 µg g⁻¹) of kg⁻¹ soil. Three replicates were maintained for each treatment. The pots were watered regularly and were maintained in open field conditions. The number of pods formed was counted after 40 days of sowing. The plants were observed for nodulation after every two days of the seedlings emergence. Five plants in each treatment were picked up randomly and nodulation characteristics like size, shape, color and number were recorded after 40 days of sowing. This period co-incide with maximum modulation in our preliminary trial on horse gram. For biochemical analysis, root nodules were collected from plants raised in different concentrations of Mo and Zn. The amount of leghaemoglobin was estimated (Tu *et al.*, 1970) after 40 days of sowing.

Statistical analysis

The mean and standard error (SE) were calculated using Microsoft Office Excel 2007. To assess the statistical significance all the values were analyzed by ANOVA (one way and two way analysis) using IBM SPSS Statistics Version 20.

Results and Discussion

The plants inoculated with the strain HGR-4 (44) had maximum number of pods at 250 µg g⁻¹ whereas the strains HGR-6 (40), 13 (45) and 25 (46) recorded at 100 µg g⁻¹ only. The number of pods formed were maximum at 250 µg g⁻¹ in control plants. Varied responses of micronutrients have been reported in literature with respect to growth and yield. Duraisamy and Mani (2001) reported that combined application of micronutrient Fe and Mo significantly increased the yield of horse gram. Mo increased pod yield in chickpea (Khanal *et al.*, 2005). In lentil (*Lens culinaris* Medic) number of pods formed per plant were more under the treatment of Mo at 235 to 248 µg g⁻¹ (Togay *et al.*, 2015) The number of pods/plant in groundnut was maximum at 250 µg g⁻¹ Mo (Deb *et al.*, 2006). At 100 mg g⁻¹, Mo increased the vegetative growth and yield of black gram compared with the control (Singh *et al.*, 2006). Mo at 500 µg g⁻¹ in soil improved plant growth and seed yield of chickpea (Johansen *et al.*, 2007). At 12 µg g⁻¹, Mo has shown promotive effect on groundnut yield parameters compared with untreated plants (Nadia Gad, 2012). Mo at 16 µg g⁻¹ increased pods yield by about 31.5% as compared to control but, above 16 µg g⁻¹ concentration it showed negative effect (Nadia Gad and Abd El-Moez, 2013). Chickpea, mung bean and groundnut have also shown significant increase in pod yield in response to Mo (Bhagiya *et al.*, 2005). It has positive effect on yield quality and nodules formation in legume crops. Mo at different concentrations significantly increased the yield of chickpea over control. Grain yield was maximum (433 mg g⁻¹) with Mo at 500 µg g⁻¹ while minimum (280 mg g⁻¹) in control for chickpea genotypes (Khan *et al.*, 2014).

In Zn treated soils too, the number of pods formed were maximum with prior inoculation of the strain HGR-25 followed by HGR-6 and HGR-13 at 500 µg g⁻¹ whereas the strain HGR-4 responded at 250 µg g⁻¹. In control uninoculated plants also the number of pods formed were maximum at 250 µg g⁻¹ (Table. 1). Desta *et al.*, 2015 also reported 36.2% increase in pod yield due to zinc application in fababean.

The number of nodules were maximum at 250 µg g⁻¹ Mo in control plants (50) and as well as the plants inoculated with the

Table 1. Mo and Zn effect on pod formation of Horse gram plants inoculated with four *Rhizobium* strains. All the values were analyzed by two way ANOVA using IBM SPSS Statistics Version 20 and were significant at 5% level of significance (LOS). *P* value < 0.05

Metal concentration ($\mu\text{g g}^{-1}$)	<i>Rhizobium</i> strains inoculated									
	Control		HGR-4		HGR-6		HGR-13		HGR-25	
	Mo	Zn	Mo	Zn	Mo	Zn	Mo	Zn	Mo	Zn
100	42	45	30	40	40	46	45	38	46	45
250	46	50	44	50	36	50	38	52	40	60
500	38	38	35	38	30	56	36	52	32	65
750	30	30	25	30	25	42	28	40	26	48
1000	30	30	20	30	20	38	22	34	21	36

strains HGR-4 (100), 6 (84) and 25 (45). But, the plants inoculated with the strain HGR-13 (50) recorded maximum at 750 $\mu\text{g g}^{-1}$ only. These values are statistically significant at 5% level of significance (*P* value < 0.05). Mo had a significant promotive effect on groundnut nodule number/plant and significantly increased number and weight of pods/plant (Nadia Gad, 2012). However, different concentration optimas have been reported in literature with respect to nodulation in different legumes. Mo at 0.05-20 $\mu\text{g g}^{-1}$ increases the number of nodules in chickpea (Verma *et al.*, 1988). Seed inoculation with Mo as well as *Rhizobium* and phosphate solubilizing bacteria (PSB) containing biofertilizers exhibited better performance than untreated control and seed treatment with Mo and biofertilizers increased nodule number and dry weight in cowpea (Chatterjee and Bandyopadhyay, 2015). Mo at 400 $\mu\text{g g}^{-1}$ increased number of nodules in cowpea while 200 $\mu\text{g g}^{-1}$ increased nodules dry weight (Alves *et al.*, 2002). Sodium molybdate (at 1.2 g g^{-1}) through priming solution and soil application of Mo at 500 $\mu\text{g g}^{-1}$ increased nodulation in both chickpea and mung bean (Khanal *et al.*, 2005). Plants treated with 500 $\mu\text{g g}^{-1}$ of Mo have formed 62.4% more nodules

when compared to control in hairy vetch roots (Alam *et al.*, 2015). Mo at 200 $\mu\text{g g}^{-1}$ seeds increased total number of peanut nodules (57.2) and number of effective nodules (27.8) than at control (Niranjana *et al.*, 2005). At 25 $\mu\text{g g}^{-1}$ Mo significantly increased the number of nodules/plant by 46.00 and 50.42 during 45 and 60 days after sowing in groundnut (Deb *et al.*, 2006). Mo applied at 500 $\mu\text{g g}^{-1}$ improved nodulation (Johansen *et al.*, 2007) and number of nodules per plant in mung bean with 200 mg g^{-1} and 1 mg g^{-1} (Bhuiyan *et al.*, 2008). Mo has positive effect on cowpea root nodules number/plant, fresh and dry weights of nodules with all Mo levels compared with control. At 16 $\mu\text{g g}^{-1}$ Mo increased both number and weight of cowpea root nodules, but above that concentration it reflected negative effect. Groundnut seeds treated with Mo at 25 $\mu\text{g g}^{-1}$ of significantly increased the number of nodules per plant (Deb *et al.*, 2006; Nadia Gad and Abd El-Moez, 2013).

In the present study the number of nodules increased with increase in Zn concentration too up to 1000 $\mu\text{g g}^{-1}$ with the strain HGR-13. Corresponding concentration for HGR-25 and HGR-4 was 750 and 500 $\mu\text{g g}^{-1}$, respectively. But, less number of nodules were observed with

Table 2. Mo and Zn effect on number of nodules of horse gram plants inoculated with four *Rhizobium* strains. All the values were analyzed by two ways ANOVA using IBM SPSS Statistics Version 20 and were significant at 5% level of significance (LOS). *P* value < 0.05.

Metal concentration ($\mu\text{g g}^{-1}$)	<i>Rhizobium</i> strains inoculated									
	Control		HGR-4		HGR-6		HGR-13		HGR-25	
	Mo	Zn	Mo	Zn	Mo	Zn	Mo	Zn	Mo	Zn
100	45	40	50	38	60	40	37	35	40	46
250	50	45	100	45	84	46	31	35	45	50
500	48	52	30	60	56	50	25	70	36	60
750	40	44	20	36	20	36	50	80	30	65
1000	32	40	20	24	22	30	44	80	23	42

the strain HGR-6. These values are statistically significant (Table 2) at 5% level of significance (P value < 0.05). The number of nodules and dry weight of nodules plant^{-1} after 30 and 45 days of sowing, increased with increasing levels of Zn up to 75 mg g^{-1} . The increase in nodulation might be due to the enhanced rooting system with the application of Zn. Application of Zn has shown good response on nodulation in blackgram also (Pavadai *et al.*, 2004).

At $250 \text{ } \mu\text{g g}^{-1}$ Mo, the amount of leghaemoglobin was maximum with prior inoculation of the rhizobial strains HGR-4 ($560 \text{ } \mu\text{g g}^{-1}$), 6 ($524 \text{ } \mu\text{g g}^{-1}$) and 25 ($496 \text{ } \mu\text{g g}^{-1}$). Rhizobial strain HGR-13 was invariably associated with lowest leghaemoglobin content irrespective of molybdenum concentration. The corresponding leghaemoglobin content was $496 \text{ } \mu\text{g g}^{-1}$ in control plants (Fig. 1). Mo is one of the essential components of nitrogenase enzyme (Burris, 1969), which have been reported to increase the leghaemoglobin content of nodules in many plants. The application of Mo stimulated nodulation and biological nitrogen fixation, thus increasing the pea yield (Brikić *et al.*, 2004). The functions of Mo in leguminous plants include nitrate reduction, nodulation, nitrogen fixation and general metabolism (Togay *et al.*, 2008). Mo is useful in formation of root nodules in urd bean (Singh *et al.*, 2006; Bhuiyan *et al.*, 2008). Mo application can play a vital role in increasing the nitrogen fixation

and nodulation in mung bean (Bhuiyan *et al.*, 2008). Mo improved nodulation in groundnut and enhanced nitrogenase activity (Tripathy *et al.*, 1999a, b). It increased the rate of symbiotic nitrogen fixation, competitive ability of peanut besides nodule number (Jing *et al.*, 2006).

Zinc application @ $500 \text{ } \mu\text{g g}^{-1}$ increased the amount of leghaemoglobin that was maximum with prior inoculation of the rhizobial strains HGR-4 ($516 \text{ } \mu\text{g g}^{-1}$) and 6 ($526 \text{ } \mu\text{g g}^{-1}$) along with control plants ($484 \text{ } \mu\text{g g}^{-1}$). The plants inoculated with the strains HGR-13 ($563 \text{ } \mu\text{g g}^{-1}$) and 25 ($502 \text{ } \mu\text{g g}^{-1}$) reflected maximum response at $750 \text{ } \mu\text{g g}^{-1}$ (Fig. 2). These values are statistically significant at 5% level of significance (P value is < 0.05). Significant increase in the leghaemoglobin content was observed due to seed treatment of *Bradyrhizobium* strains along with different micronutrients. Soil application of Zn @ 5 mg g^{-1} and ammonium molybdate @ 1 mg g^{-1} increased the nodule number in soybean (Chandel *et al.*, 1989). Zn has been reported as essential for synthesis of indole acetic acid (IAA) (Nason *et al.*, 1951). The increased IAA due to Zn increased the nodule number (Soundararajan *et al.*, 1985). Mo and Zn which are the metallic components of one or more enzymes are involved in various physiological functions, growth, development and productivity of the plant. Hence, positive response of their application with respect to nodulation in our study too.

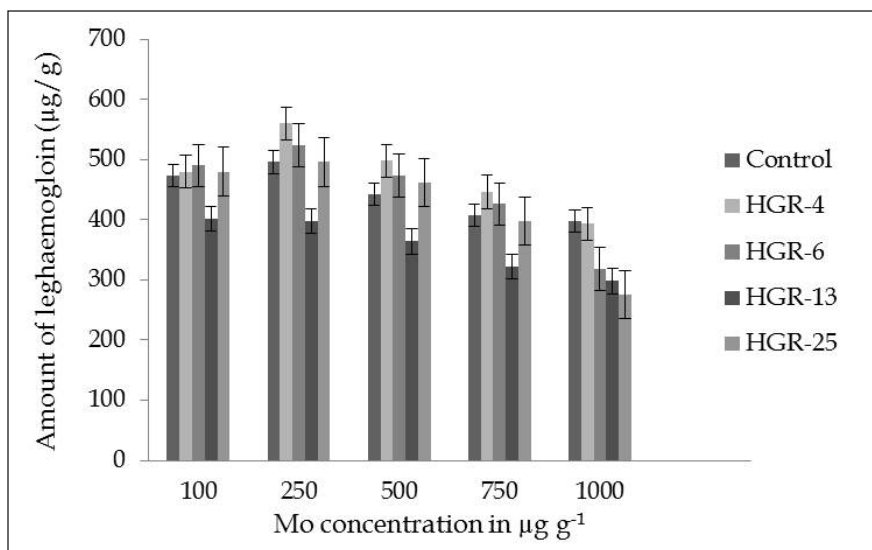


Fig. 1. Molybdenum effect on leghaemoglobin content of horse gram plants inoculated with rhizobia. Bars indicate mean of \pm SE. All the values were analyzed by ANOVA using IBM SPSS Statistics Version 20 and were significant at 5% level of significance (LOS). P value < 0.05 .

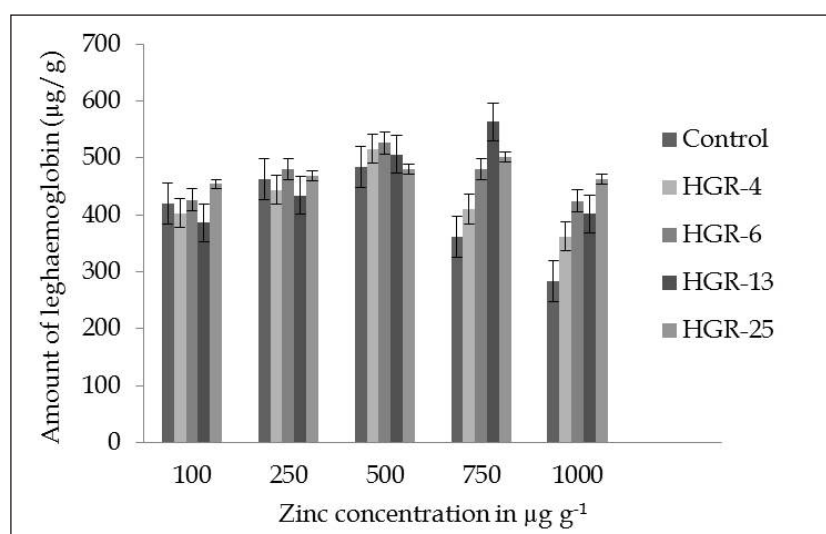


Fig. 2. Zinc effect on leghaemoglobin content of horse gram plants inoculated with rhizobia. Bars indicate mean of \pm SE. All the values were analyzed by ANOVA using IBM SPSS Statistics Version 20 and were significant at 5% level of significance (LOS). P value <0.05 .

The increase in nodulation at low levels of Zn was associated with its role in auxin production through tryptophan synthesis (Burstorm, 1951) while decrease of high levels might be attributed to the interference in the nutrition of the host as well as *Rhizobium* (Shukla and Yadav, 1982). Nitrogen fixation increase has also been reported in soybean with $10 \mu\text{g g}^{-1}$ Zn (Kapoor *et al.*, 1975). Inhibition/reduction in nodulation and N fixation may be due to Zn deficiency and/or toxicity leading to interference in nutrition of host and/or *Rhizobium* (Demeterio *et al.*, 1972). At higher concentration Zn reflected negative effect on nodule weight, while N addition has also been reported to completely inhibit nodulation (Cusato *et al.*, 2007). The fertility level F_2 (60-40-5, P_2O_5 -S-Zn kg ha^{-1}) was found most effective in enhancing the plant growth, nodulation, yield, nutrient content in nodules and nutrient uptake by pea (Singh *et al.*, 2014).

The plants inoculated with the strains HGR-6, 13 and 25 have formed more pods at $100 \mu\text{g g}^{-1}$ while the amount of leghaemoglobin formed was maximum ($560 \mu\text{g g}^{-1}$) at $250 \mu\text{g g}^{-1}$ with the strain HGR-4 in Mo containing soils. In case of zinc, the amount of leghaemoglobin was maximum ($563 \mu\text{g g}^{-1}$) at $750 \mu\text{g g}^{-1}$ of Zn when the plants were inoculated with the strain HGR-13 and the number of pods formed were high with the strains HGR-6 and HGR-13 at $500 \mu\text{g g}^{-1}$. Thus, the present study clearly demonstrates that horse gram plants in soils having high

concentration of Mo and Zn in the range of 100 - $1000 \mu\text{g g}^{-1}$ soil would effectively nodulate and yield by using proper strain of *Rhizobium*.

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References

- Alam, F., Kim, T.Y., Kim, S.Y., Alam, S.S., Pramanik, P., Kim, P.J. and Lee, Y.B. 2015. Effect of molybdenum on nodulation, plant yield and nitrogen uptake in hairy vetch (*Vicia villosa* roth). *Soil Science and Plant Nutrition* 61: 664-675.
- Alves, J.M., Guimaraes, E.C. and Alves, J.S. 2002. Molybdenum application in cowpea [*Vigna unguiculata* (L.) Walp] leaf. *Revista Universidade Rural. Serie Ciencias da Vida* 22: 193-197.
- Bhagiya, S.R., Polara, K.B. and Polara, J.V. 2005. Effect of B and Mo on yield, quality and nutrient absorption by groundnut. *Advances in Plant Sciences* 18: 803-806.
- Bhuiyan, M.M.H., Rahman, M.M., Afroze, F., Sutradhar, G.N.C. and Bhutyan, M.S.I. 2008. Effect of phosphorus, molybdenum and *Rhizobium* inoculation on growth and nodulation of mung bean. *Journal of Soil and Nature* 2: 25-30.
- Brikić, S., Milaković, Z., Kristek, A. and Antunović, M. 2004. Pea yield and its quality depending on inoculation, nitrogen and molybdenum fertilization. *Plant Soil and Environment* 50: 39-45.
- Burris, R.H. 1969. Progress in the biochemistry of nitrogen fixation. *Proceedings of the Royal Society London* 8, 172: 339-354.

- Burstorm, S.M. 1951. In *Plant Growth Substances*, pp. 340. Univ. Wisconsin, Madison, USA.
- Chatterjee, R. and Bandyopadhyay, S. 2015. Effect of boron, molybdenum and biofertilizers on growth and yield of cowpea (*Vigna unguiculata* L. Walp.) in acid soil of eastern Himalayan region. *Journal of the Saudi Society of Agricultural Sciences* doi:10.1016/j.jssas.2015.11.001.
- Chandel, A.S., Tiwari, S.K. and Saxena, S.C. 1989. Effect of micronutrient application on soybean (*Glycine max.*) growth in Uttar Pradesh foothills. *Indian Journal of Agricultural Science* 59: 62-63.
- Cusato, M.S., Tortosa, R.D., Valiente, L., Barneix, A.J. and Puelles, M.M. 2007. Effects of Zn²⁺ on growth and nodulation of a South American actinorhizal plant *Discaria americana* (Rhamnaceae). *World Journal of Microbiology and Biotechnology* 23: 771-777.
- Deb, R., Poddar, P. and Banerjee, S. 2006. Effect of various modes of application of molybdenum on nodulation yield and economics of groundnut cultivation under terai region of West Bengal. *Environment and Ecology* 24: 1081-1084.
- Demeterio, J.L., Jr. Roscoe, E. and Poulsen, G.M. 1972. Nodulation and nitrogen fixation by two soybean varieties as affected by phosphorus and zinc nutrition. *Agronomy Journal* 64: 566-568.
- Desta, Y., Habtegebrial, K. and Weldu, Y. 2015. Inoculation, phosphorus and zinc fertilization effects on nodulation, yield and nutrient uptake of Faba bean (*Vicia faba* L.) grown on calcareous cambisol of semi-arid Ethiopia. *Journal of Soil Science and Environmental Management* 6: 9-15.
- Duraisamy, P. and Mani, A.K. 2001. Effect of iron and molybdenum on yield and nutrition of horse gram in red loamy sand soil. *Mysore Journal of Agricultural Science* 35: 26-31.
- Jing Li, Y., Xiao Ping, Z., Deng Yu, L., Qiang, C. and KeMei, W. 2006. Study of the effects of Mo and *Brady rhizobium* inoculation in acid purple soil by celB gene marker. *Plant Nutrition and Fertilizer Science* 12: 250-253.
- Johansen, C., Musa, A.M., Rao, J.V.D.K.K., Harris, D., Ali, M.Y., Shahidullah, A.K.M. and Lauren, J.G. 2007. Correcting molybdenum deficiency of chickpea in the High Barind Tract of Bangladesh. *Journal of Plant Nutrition and Soil Science* 170: 752-761.
- Khan, N., Tariq, M., Ullah, K., Muhammad, D., Khan, I., Rahatullah, K., Ahmed, N. and Ahmed, S. 2014. The effect of molybdenum and iron on nodulation, nitrogen fixation and yield of chickpea genotypes (*Cicer arietinum* L.). *IOSR Journal of Agriculture and Veterinary Science* 7: 63-79.
- Khanal, N., Joshi, K.D., Harris, D. and Chand, S.P. 2005. Micronutrients in South and South East Asia: Proceedings of an International Workshop held in Kathmandu, 8-11 Sept., pp. 121-132, Nepal.
- Kapoor, D.C., Gangwar, M.S. and Tilak, K.V.B.R. 1975. Influence of zinc on symbiotic nitrogen fixation by soybean (*Glycine max.* L.) in silt loam soil. *Indian Journal of Agricultural Research* 9: 51-56.
- Marsh, D.B. and Waters, Jr. L.J. 1985. Critical deficiency and toxicity levels of tissue zinc in relation to cowpea growth and N₂ fixation. *American Society Horticultural Science* 110: 365-370.
- Meagher, W.R., Johnson, M. and Stout, P.R. 1991. Molybdenum requirement of leguminous plants supplied with fixed nitrogen. *Plant Physiology* 27: 623-629.
- Nadia Gad 2012. Influence of molybdenum on groundnut production under different nitrogen levels. *World Journal of Chemistry* 7: 64-70.
- Nadia Gad and Abd El-Moez, M.R. 2013. Influence of molybdenum on nodulation, nitrogen fixation and yield of cowpea. *Journal of Applied Science Research* 9: 1498-1504.
- Nason, A., Kaplan, N.O. and Colowick, S.P. 1951. Changes in enzymatic constitution in zinc deficient *Neurospora*. *Journal of Biology and Chemistry* 188: 397-406.
- Niranjana, H.G., Prakash, S.S., Basavegowda, N.A., Yelladhalli, M. and Chandranath, H.T. 2005. Effect of micronutrient seed treatment on growth and yield of groundnut. *Seed Research* 33: 138-141.
- Pavadai, P., Dhanavel, D., Vijayarengan, P., Seetharaman, N. and Selvaraju, M. 2004. Efficacy of zinc on germination, seedling growth and biochemical contents of blackgram [*Vigna mungo* (L.) Hepper. Var. CO₃]. *Plant Archives* 4: 475-478.
- Shanti, M., Babu, B.P., Prasad, B.R. and Minhas, P.S. 2008. Effect of zinc on black gram in rice-blackgram cropping system of coastal saline soils. *Legume Research* 31: 79-86.
- Shukla, U.C. and Yadav, O.P. 1982. Effect of phosphorus and zinc on nodulation and nitrogen fixation in chickpea (*Cicer arietinum* L.). *Plant and Soil* 239-248.
- Smith, F.W. 1982. Mineral nutrition of legumes. In *Nitrogen Fixation in Legumes* (Ed. J.M. Vincent), pp. 155-172, Academic Press, Sydney, Australia.
- Singh, R.P., Singh, R.K., Yadav, P.K., Singh, S.N., Prasad, L. and Singh, J. 2006. Effect of sulphur and molybdenum on yield and quality of black gram (*Vigna mungo* L.). *Crop Research* 32: 336-338.
- Singh, D.K., Singh, A.K., Mandhata, S., Zulutemjen, J. and Srivastava, O.P. 2014. Effect of fertility levels and micronutrients on growth, nodulation, yield and nutrient uptake by pea (*Pisum sativum* L.). *Legume Research* 37: 93-97.

- Soundararajan, M.S., Sudhakara Rao, R. and Raja Rao, J. 1985. Nodulation in Spanish groundnut as influenced by secondary (Ca and 3) and micro (Mo and 9) nutrient fertilization. *Oil Seeds Journal* 15: 39-41.
- Togay, Y., Togay, N. and Dogan, Y. 2008. Research on the effect of phosphorus and molybdenum applications on the yield and yield parameters in lentil. *African Journal of Biotechnology* 7: 1256-1260.
- Togay, N., Togay, Y., Erman, M. and Cig, F. 2015. Effect of Fe (iron) and Mo (molybdenum) application on the yield and yield parameters of lentil (*Lens culinaris Medic.*). *Legume Research* 38: 358-362.
- Tripathy, S.K., Patra, A.K. and Samui, R.C. 1999a. Effect of micronutrients on nodulation, growth, yield and nutrient uptake of summer groundnut (*Arachis hypogaea*). *Annals of Agricultural Research* 20: 439-442.
- Tripathy, S.K., Patra, A.K. and Samui, R.C. 1999b. Effect of micronutrients on nodulation, growth, yield and nutrient uptake of summer groundnut (*Arachis hypogaea*). *Indian Journal of Plant Physiology* 4: 207-209.
- Tu, J.C., Ford, R.E. and Garu, C.R. 1970. Some factors affecting the nodulation and nodule efficiency in soyabeans infected by soyabean mosaic virus. *Phytopathology* 60: 1653-1656.
- Verma, L.P., Ram, P.C. and Maurya, B.R. 1988. Response of chickpea to phosphorus and molybdenum of eastern Uttar Pradesh, India. *International Chickpea Newsletter* 18: 31-33.
- Williams, R.J.P. and Frausto da Silva, J.J.R. 2002. The involvement of molybdenum in life. *Biochemistry and Biophysical Research Communication* 292: 293-299.

