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Evaluation of combined effect of micronutrients and rhizobial inoculation on mungbean productivity

Monika, Priyanka, Rajesh Yadav[1], Leela Wati

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ABSTRACT

Micronutrients play an important role in increasing yield of pulses and oilseed legumes through their effects on the plant itself and on the nitrogen fixing symbiotic process. Indian soils have become deficient not only in major plant nutrients like (NPK) but also scarce in some micronutrients like zinc, iron, molybdenum and boron. These situations suggest the need of application of micronutrients along with biofertilizers under intensive cultivation of legumes as it is directly involved in biological nitrogen fixation through nitrogenase enzyme activity. In the present research investigation, different micronutrients iron, molybdenum and their combination in the form of ferrous sulphate, ammonium molybdate and ferrous sulphate+ammonium molybdate @2.0, 2.0 and 2.5 kg/h are added into micronutrient deficient soil for plant growth promotion of mungbean under pot house condition. Among various treatments, ammonium molybdate application @2.0kg/h along with rhizobial isolate HSR1 and PSB increases nodulation (28.6%), plant biomass (18.5%), seed yield (41%) as compared to sole application of biofertilizer.

Key words: Micronutrients, Molybdenum, Mungbean, Plant growth promotion, Rhizobia, Yield.

INTRODUCTION

The use of rhizobia as the potential alternative for chemical fertilizers has gained importance worldwide. The growth promoting effects of rhizobia as plant growth promoting rhizobacteria (PGPR) have been reported in the literature in number of crops. The inoculants markedly increased nodule number, nodule mass, shoot weight and crop yield in comparison to urea treated plants. In other crops like groundnut, lentil and mungbean grain yield was increased to 30-47% as compared to control on rhizobial inoculation (Ndakidemi *et al.,* 2007).

Therefore a balance in micronutrient condition is also necessary in soil for higher crop productivity. Micronutrients play an important role in increasing yield of pulses and oilseed legumes through their effects on the plant itself and on the nitrogen fixing symbiotic process (Paliya *et al.,* 2014). The quantum and quality of *Rhizobium* is a very basic requirement to enhance the production of food legumes in Asian continent, especially in India. The common medium used for culturing rhizobia is yeast extract mannitol medium, which is made up of yeast extract powder or paste and is found to be best suited nutrient medium for *Rhizobium* growth. The ideal medium contains mannitol as the source of nitrogen, growth factors and mineral salts. Other than these elements for the appropriate growth of rhizobia, micronutrients are also essential. The small quantity of micronutrients can trigger the growth enhancement in production. Due to prolonged and overuse of chemical fertilizers in soil to increase productivity has resulted in depletion of micronutrients from soil. Micronutrient deficiency in soil results in decrease in crop productivity as well as increased malnutrition condition on consumption (Ryan *et al.,* 2013).

Legumes, which develop a symbiosis with nitrogen-fixing bacteria, have an increased demand for iron. Iron deficiency can affect both the legume host and the rhizobia individually or can have a direct effect on their interaction (Kihara *et al.,* 2016, Kihara *et al.,* 2017). Iron plays an important role in the formation of nodule proteins like nitrogenase and leghaemoglobin. Further, bacteroids have a very high respiratory demand, requiring abundant cytochromes and other electron donors, each with their own Fe centers. Iron deficiency affects shoot growth, nodule mass and particularly leghemoglobin content, number of bacteroids and nitrogenase activity (Delgado *et al.,* 1998; Delgado *et al.,* 2006). Molybdenum is also known to be a key element required by the microorganisms for nitrogen fixation. It is a constituent of nitrogenase and nitrate reductase enzymes. The nitrate reductase is essential in the assimilation of nitrates since it catalyses the first step of the reduction of NO_3 to NH_3. The other major molybdo-protein of plants includes nitrogenase, which fixes atmospheric nitrogen to NH_3, which is assimilated by plants (Adesoji *et al.,* 2009, Afloabi *et al.,* 2014). The FeMoCo cofactor is at

Department of Microbiology, Chaudhary Charan Singh Haryana Agriculture University, Hisar-125 004, Haryana, India.
[1]Department of Genetics and Plant Breeding, CCS Haryana Agriculture University, Hisar-125 004, Haryana, India.

Corresponding Author: Monika, Department of Microbiology, Chaudhary Charan Singh Haryana Agriculture University, Hisar-125 004, Haryana, India. Email: monikabarkodia90@gmail.com

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the heart of the nitrogen reduction process for most of the nitrogenases. The Mo–Fe protein contains two atoms of molybdenum and has oxidation–reduction centers of two distinct types: two iron–molybdenum cofactors called FeMoCo and four Fe-S (4Fe-4S) centers. The Fe–Mo cofactor (FeMoCo) of nitrogenase constitutes the active site of the molybdenum-containing nitrogenase protein in N_2-fixing organisms. Molybdenum deficiency in soil induced nitrogen deficiency in legumes relying on N_2 fixation (Anju *et al.,* 2015; Weisany *et al.,* 2013).

Thus, it is necessary to examine the effect of different levels of micronutrients on symbiotic efficiency of rhizobia and productivity of mungbean. The aim of this study is to evaluate the combined effect of micronutrients and rhizobial inoculation on mungbean productivity.

MATERIALS AND METHODS

Rhizobial strain HSR1 used in this study was *Sinorhizobium sp* strain isolated previously during our study (Monika, 2017). Phosphate solubilizing bacterial strain used in this study was commercial fertilizer *Pseudomonas* P36 of biofertilizer production unit, CCSHAU, Hisar. For inoculum preparation, a loopful of culture from freshly prepared slants of rhizobial isolates was transferred to YEM broth. The rhizobial isolates were grown in YEM broth for three days on shaker at 30°C.Effect of various micronutrients on growth of rhizobia was evaluated after three days of incubation. One ml of the inoculum was transferred to the pre-sterilized yeast extract mannitol broth (YEMB) containing different concentration (0.05, 0.1, 0.5, 1.0 and 1.5%) of micronutrients (Fe, Mo, B and Zn) followed by incubation at 28-30°C and initial population was estimated by using standard plate count technique. Viable count of rhizobia in micronutrient (Fe, Mo, B and Zn) supplemented YEM broth was taken from 1st to to 7th day of incubation on YEMA medium with congo-red as described by (Vincent 1970). Culture (0.1ml) of inoculated rhizobial isolate was spread plated on YEMA plates. Three replications were maintained for each treatment. The plates were incubated at 28°C for 3 days. After 3 days of incubation, rhizobial colonies on YEMA medium plates were counted manually. The population was expressed as log cfu/ml.

The effect of selected micronutrient and mungbean rhizobia was studied on the growth and yield of mungbean variety MH 421 in the year 2016 (Summer Season). Mungbean seeds were treated with full grown culture broth of selected rhizobial isolates and sown in pots (12 inch) containing 5kg of unsterilized soil in triplicate under pot house conditions. Recommended dose of phosphorus (60 kg) in the form of di-ammonium phosphate (DAP) and nitrogen (120kg) in the form of urea was applied. Analysis of pot house soil revealed that it contained iron 4mg/kg and molybdenum 2.0 mg/kg.Therefore, in pot house experiment, some treatments were made with the supplementation of micronutrients i.e. ferrous sulphate, ammonium molybdate and combination of ferrous sulphate+ ammonium molybdate (@2.0, 2.0, 2.5 kg/ha) to check the effect of micronutrient supplementation on mungbean productivity and performance of rhizobia. Uninoculated seeds were kept as control and three plants per pot were maintained. Plants were irrigated with tap water as when required. Plants were uprooted at maturity of the crop and observations on plant height, shoot weight, root weight and seed yield were recorded.

Statistical analysis was done by using OPSTAT data analysis tool with application of complete randomized design (CRD) with 3 replicates. A 5% probability level (p=0.05) was used to accept or reject null hypothesis.

RESULTS AND DISCUSSION

In the previous study (Monika, 2017), the rhizobial isolate HSR1 (*Sinorhizobium sp* HSR1) was isolated from mungbean root nodule showed promising plant growth promoting traits (IAA production, ACC deaminase activity, ammonia excretion, HCN production, siderophore production) was used as rhizobial strain. Commercial biofertilizer *Pseudomonas striata* (P36) strain was used as PSB in this study.

In the present study, effect of different concentrations of iron, molybdenum, boron and zinc in the form of ferrous sulpahte, sodium molybdate, sodium tetraborate (borax) and zinc sulphate on growth of rhizobial isolate HSR1 was evaluated. On the evaluation of the effect of micronutrient different concentration on rhizobial isolate HSR1 had maximum log cfu/ml on supplementation of 1% ammonium molybdate in YEM broth. It was found that combination of micronutrients (0.5% ferrous sulphate + ammonium molybdate) was found to be the best in terms of increase in log cfu/ml followed by 1% concentration of ferrous sulphate and ammonium molybdate. It was found that 0.5% ferrous sulphate + ammonium molybdate concentration had 34.9%, 1% ferrous sulphate had 31% and 1% ammonium molybdate had 25% more log cfu/ml as compared to control (Table 1). Therefore, to increase the efficiency of rhizobia, rhizobial strain HSR1 was grown in YEM broth supplemented with different concentration of micronutrients i.e. 1% ferrous sulfate, 1% ammonium molybdate and 0.5% combination of ferrous sulpahte and ammonium molybdate (1:1 ratio).These results are in consistent to the reports that the combination of low concentration of iron and molybdenum in YEM broth accelerate growth rate of *Rhizobium* (Goudar *et al.,* 2008, Gauri *et al.,* 2012; Bansod and Upadhayay, 2014).

Nodulation is an important parameter in legume plants growth. On the basis of performance of various treatments in pot house experiment it was observed that on individual application of micronutrients increases nodule number but increase in nodule number is less as compared to sole application HSR1 and PSB but it higher than RDF. Application of micronutrients along with rhizobial isolate and PSB significantly increases nodule number of mungbean plant (Table 2). On application of micronutrients along with biofertilizers nodule number increased (varied from 35.0 to

Table 1: Effect of different concentrations of micronutrients on growth of mungbean rhizobial isolate HSR1.

Days	*Control (YEMB)	Log cfu ml^{-1} $FeSO_4$ concentration (%)			$(NH)_4$ Mo_7cocentration (%)			$FeSO_4$+ $(NH)_4$ Mo_7concentration (%)		
		0.5	1.0	1.5	0. 1	0.5	1.0	0.1	0.5	1.0
1	2.267	3.591	3.591	2.505	3.806	4.361	3.477	3.653	3.799	3.079
2	3.298	4.688	5.089	3.623	4.944	6.531	3.505	5.123	5.161	4.964
3	5.368	7.509	6.621	7.082	6.735	7.643	5.819	6.225	7.828	6.610
4	6.431	7.982	8.191	8.103	8.093	8.238	7.819	6.999	9.086	7.791
5	8.380	9.099	10.968	8.402	9.995	10.481	8.792	9.492	11.225	9.497
6	7.260	8.663	9.438	7.477	7.663	7.995	7.690	7.318	8.619	9.653
7	7.079	7.531	8.477	7.301	7.298	7.447	7.447	7.131	7.278	7.568

Table 2: Effect of micronutrients applications along with biofertilizers (HSR1+PSB) on nodule number, dry weight, plant height, seed number, seed yield of mungbean under pot house condition (2016).

Treatments	Nodule number /plant	Dry weight (g/plant)	Plant height	Seed number (mg/plant)	Seed yield g/plant
Control	15.6	1.16	18.0	17.3	0.78
RDF	19.8	1.50	19.0	26.0	1.04
*Ferrous sulphate	21.5	1.69	19.2	30.6	1.44
*Ammonium molybdate	25.7	1.75	20.5	34.0	1.66
*Ferrous sulphate+ Ammonium molybdate	23.8	1.70	19.6	32.0	1.55
HSR1	29.4	2.40	22.0	44.6	2.22
PSB	21.6	1.88	20.0	35.0	1.76
HSR1+ PSB	32.1	2.70	25.0	50.6	2.44
HSR1+ **Ferrous sulphate (1%)	33.4	2.67	24.6	57.6	2.40
HSR1+ **Ammonium molybdate (1%)	35.3	2.96	25.9	63.6	2.92
HSR1+ **Ferrous sulphate + Ammonium molybdate (0.5%)	34.6	2.78	26.6	75.0	2.66
HSR1+ Ferrous sulphate (1%) + PSB	35.0	2.79	26.6	65.0	2.66
HSR1+ **Ammonium molybdate (1%) + PSB	41.3	3.20	28.9	84.0	3.46
HSR1+ ** Ferrous sulphate+ Ammonium molybdate (0.5%) + PSB	36.9	2.86	27.8	73.0	2.98
C.D. at 5%	1.526	0.062	0.08	1.2	0. 21

*=Soil application, **=Soil application + amended in YEMB.

41.3 nodules /plant) as compared to control and individual application of biofertilizers. The highest number of nodules (41.3 nodules/plant) was recorded on the application of HSR1+ ammonium molybdate (@ 2 kg/h)+PSB followed by application of HSR1+ferrous sulphate + ammonium molybdate (0.5%)+PSB (36.9 nodules/plant). Coinoculation of ammonium molybdate with HSR1 and PSB increases nodule no to 41% as compared to sole inoculation of HSR1 and 28.6% increased nodulation as compared to coinoculation of HSR1+PSB. Therefore, seeds inoculated with rhizobial isolate HSR1, PSB, and treatment ammonium molybdate had statistically significant effect on nodule count of plant. Increase in nodule number and nodule weight due to the application of micronutrients and biofertilizers might be due to enhanced activity of *Rhizobium* due to supplementation of micronutrients *viz*. Mo, Fe, which are very essential for nitrogenase activity for biological nitrogen fixation by N_2-fixing bacteria. These findings were in agreement with those reported by Gupta and Sahu, (2012). Application of molybdenum recorded maximum nodule number and nodule weight suggesting that application of Mo allowed synthesis of more nodule tissue due to better supply of Mo from soil to plants and also by maintaining supply of essential metabolites to the nodules. Togay *et al.,* (2008) found that seed treatment with Mo has a positive effect on growth, yield parameters including number of pod bearing branches in legume crops. There was highly significant nodulation response of mungbean plants treated with molybdenum and seed inoculation. Similar results were reported by Das *et al.,* (2012) that chickpea treated with molybdenum and biofertilizers, achieved significantly higher nodule number as well as nodule leghaemoglobin. Chatterjee and Bandyopadhyay (2015) also observed similar results on micronutrient application with biofertilizers.

Dry weight increased on application of micronutrients along with biofertilizers (HSR1+PSB). Maximum dry weight i.e. 3.20 g/plant was recorded on application of ammonium molybdate in soil along with rhizobial isolate HSR1 (grown

in YEMB supplemented with 1% ammonium molybdate) and PSB, followed by application of ferrous sulphate+ammonium molybdate+HSR1+PSB *i.e.* 2.86 g/plant (Table 2). It is because molybdenum is an essential component of nitrogenase enzyme responsible for BNF through *Rhizobium*. Maximum plant dry weight 3.2g/plant was observed on application of ammonium molybdate (@2.0kg/h) along with rhizobial isolate HSR1and PSB. Application of biofertilizers (HSR1+PSB) along with ammonium molybdate showed 33% higher plant dry weight as compared to individual inoculation of HSR1, 18.5% higher dry weight as compared to coinoculation of HSR1+PSB. Increased N_2 fixation through Mo application along with *Rhizobium* inoculation might have resulted better growth and more uptake. Johansen *et al.,* (2007) found that seeds treated with Mo had greater total dry matter production. Awomi *et al.,* (2015) obtained maximum growth and yield with application of 1.5 kg Mo/ha. Such increase in plant dry weight due to Mo application was also reported by Poonia and Pithia (2014).

Application of micronutrients (soil application) along with biofertilizers (rhizobial isolate grown in YEM broth supplemented with micronutrients) increased seed yield effectively as compared to their individual inoculation. Maximum seed yield 3.46 g/plantwas obtained using ammonium molybdate as a micronutrient with rhizobial isolate HSR1 and PSB, followed by application of ferrous sulphate + ammonium molybdate +HSR1+ (2.98g/plant). Application of biofertilizers (HSR1+PSB) along with ammonium molybdate (@2.0kg/h) showed 55% higher seed yield as compared to individual inoculation of HSR1 and 41% higher seed yield as compared to combined inoculation of HSR1+PSB (Table 2). Seed yield is highly dependent on plant height and nodulation count. Higher yield from the treatment *Rhizobium*+PSB+Mo was a reflection of high nitrogen supply due to better nodulation when inoculated with Mo and biofertilizers resulting in high nitrogen fixation, better plant growth and yield contributing parameters (Rahman *et al.,* 2008). Togay *et al.* (2015) also observed increased seed yield in chickpea on application of iron and molybdenum. Similar observations were reported by Tahir *et al*. (2011) and Rechiatu *et al*. (2015) of chick pea and soybean respectively.

We obtained maximum nodulation, plant height, seed number, seed yield and plant biomass on application ammonium molybdate @ 2.0 kg/h with coinoculation of biofertilizers. Ammonium molybdate @ 2.0 kg/h with coinoculation of biofertilizers increases nodulation (28.6%), plant biomass (18.5%) and seed yield (41%) as compared to sole application of biofertilizer. These results were in agreement with Tiwari *et al.,* (2018). Nasar and Shah (2017) suggested that combined application of iron @ 1.0 kg ha^{-1} and molybdenum @ 0.10 kg ha^{-1} as foliar spray significantly enhanced the crop yields and root nodulation in lentil under the agro-climatic conditions of Swat valley. In present study yield was less on coinoculation of iron and molybdenum @2.5kg/h, it was because higher conc. of molybdate and iron in soil, sometimes higher nutrient concentration had negative effect on yield due to salt stress or heavy metal stress. But our study inclined with the finding of different scientists. Tahir *et al.,* (2011) obtained greater economic benefit when the mungbean seed is treated with the Mo at the rate 4 g/kg of seed + inoculated with *Rhizobium*. Kumar and Sharma (2005) also reported same increase in yield with Mo application in soil with biofertilizers.

CONCLUSION

Micronutrient plays an important role in growth and yield of crops. It was proved from this research investigation that the soil application of molybdenum in soil as ammonium molybdate along with biofertilizers (*Rhizobium*+PSB) was found to be very effective in increasing the growth and grain yield in mungbean plant grown in soil deficient in molybdenum. Nodulation, plant height, seed weight, seed yield and plant biomass were enhanced significantly on application of ammonium molybdate in soil with inoculation of biofertilizers. Ammonium molybdate application along with rhizobial isolate HSR1 can be further assessed for their efficiency under field conditions. This indicates the necessity of micronutrient amendment with combination of biofertilizers (*Rhizobium* and PSB) especially in area under mungbean cropping sequence to evaluate the Mo status and to decide the Mo application wherever necessary.

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