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EFFECT OF DIFFERENT LEVELS OF MOLYBDENUM AND *RHIZOBIUM PHASEOLI* IN RICE-MUNG BEAN CROPPING SYSTEM

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Abstract

Ignorance of trace elements (iron, cobalt and molybdenum etc.) may result in reduced crop yield. Leguminous crops are very sensitive towards molybdenum (Mo) deficiency, but excessive Mo may also impair the growth, decrease biomass, seed yield and quality of produce. Many scientists had documented information regarding sole inoculation of combined *Rhizobium* and effects of Mo, however, limited information is available on combined use of Mo and *Rhizobium* inoculation on mung bean (*Vigna radiata* L.) productivity. Therefore, current study was conducted for assessing the effects of *Rhizobium* with Mo supply on yield attributes and N_2-fixation in *V. radiata*. There were 2 levels of *Rhizobium* inoculations (with and without rhizobia) and 5 levels of Mo (0, 3, 4, 5 and 6 g kg^{-1} seed) applied during 2015 and 2016 cropping seasons for cultivation of *V. radiata*. Results showed that at maturity plant height, pod bearing branches/plant, nodules and pods/plant, seeds/pod, pod length, 1000-grain weigh, seed yield and protein were significantly increased 25.4, 80.7, 109.3, 22.3, 49.4, 43.3, 23.6, 96.9 and 18.9 % respectively, where Mo (4 g/kg) + *R. phaseoli* was applied as compared to control. In conclusion, combined use of Mo and *Rhizobium* have potential to enhance productivity and quality of mung bean.

Key words: Growth attributes, Mungbean, Mo, *Rhizobium phaseoli*, Yield attributes.

Introduction

Molybdenum (Mo) is a trace element found in the soil and is required for optimum growth of plants. Despite its low availability, Mo ranks 53rd in the earth's crust. It is more available to biological processes comparative to other metals, that are found in chemical forms and are difficult to assimilate (Hernandez *et al.,* 2009). Molybdenum itself is not biologically active but is predominantly found to be an integral part of an organic pterion complex called the Mo co-factor (Moco). This co-factor Moco has ability to bind Mo requiring enzymes i.e., molybdoenzymes that is found in most biological systems (plants, animals and prokaryotes). Similarly, o-nitrogenase is responsible for most biological nitrogen fixation process that determines the global biogeochemical cycles of nitrogen and carbon (Williams & Frausto da Silva, 2002).

When plants are cultivated under Mo deficient environment, the growth of plants become restricted due to phenotypes. These phenotypes are usually associated with reduced activity of molybdoenzymes that is involved in the primary nitrogen assimilation enzymes such as nitrate reductase (NR) and the nitrogen-fixing enzyme nitrogenase. That's why role of Mo is considered positive on growth, yield, nodule forming, N content of roots and foliage in legume crops (Yesim *et al*., 2008). In addition to above, it is also involved in plant nutrition especially in nitrogen metabolism that improved quality of soluble sugar, chlorophyll and ascorbic acid (Zhao & Bai, 2001; Chen & Nian, 2004). Similarly, Mo deficiency may decrease pods, plant growth and grain development (Graham & Stangoulis, 2005).

On the other hand, the importance of biological nitrogen fixation (BNF) is unquestioned especially in underdeveloped countries, sustainable agriculture and world food security. However, in different soils, *Rhizobium* species population may vary which mostly remained less than required population for BNF. It is well established that *Rhizobium* inoculation improve the nodulation and nitrogen fixation in the legumes (Kevin, 2004). Isolation of specific bacterial species and their augmentation is considered an effective way to improve the Rhizobial population. These *Rhizobium* species usually remain sessile in the rhizosphere and root hairs where they played an imperative role in nodule formation and N fixation. Similarly, supplying of Mo may alsp facilitate nodulation, nitrogen uptake and seed protein formation in legumes (Bejandi *et al*., 2012). So far beneficial effects of Mo as well as *Rhizobium* inoculation on crops growth are widely investigated (Staley and Brauer, 2006) but missing in tropical and sub-tropical regions of Pakistan. By utilizing rhizobial strains as biofertilizer for sustainable production of cereal crops under water deficit condition (Hussain *et al*., 2018).

In addition to above, cultivation of leguminous cropping patterns, can also improve the yield of rice comparative to conventional rice-wheat cropping pattern (Ali *et al*., 2012). Legume-rice rotation can also improve soil N status (Peoples *et al*., 2009) and soil organic matter has great impact on water holding capacity (Libohova *et al*., 2018). Among all, mung bean (*Vigna radiata* L.) is an important leguminous crop which is widely grown in Pakistan. Highly palatable, digestible, and its nutritive values i.e., iron = 7.3, zinc = 3, calcium = 124 and foliate = 549 mg / 100 g of dry seed make it an important crop among all pulses. According to a survey 163.2 thousand hectares of land in Pakistan is under mung bean cultivation that produce yield of 117.8 thousand tons (Anon., 2018-19). However, growth and yield of mung bean could be improved via inoculation of Rhizobia, documented as an alternate of N fertilizers (Ndakidemi *et al*., 2006).

Scientific justification is necessity of time, required to support the expanding legme cultivation with the inoculation of *R. phaseoli* in those areas where soil Mo profiles limit plant growth and productivity. Thus, the main objective of current study was to compare different levels (0, 3, 4, 5 and 6 g kg^{-1} seed) of Mo for improving the efficacy of *R. phaseoli* in mung bean especially in cropping system of rice-mung bean. The specific objectives were to assess the effects of *Rhizobium* and Mo on growth, nitrogen fixation, yield and protein and chlorophyll contents in *V. radiata*.

Materials and Methods

Site selection and description: Experiment was conducted in Agronomic Research Station (31.25°N, 73.09°E, and 184.82 m asl), University of Agriculture, Faisalabad, Pakistan, during 2015 and 2016 growing seasons. Soil of experimental site was characterized as Lyallpur soil series, Aridisol-fine silty, Haplic Yermosols in FAO classification scheme and Haplargid in USDA. Previously rice was grown on the experimental area.

Seeds: Mung bean cultivar AZRI-2006 seeds were collected from the Ayub Agriculture Research Institute (ARRI), Faisalabad.

Source of Mo and Rhizobium: Sodium molybdate was used as Mo source which was obtained from Abdullah Scientific Traders, Faisalabad. Twelve hours before planting, seeds were soaked into Mo solution. The control was also soaked in a water solution containing zero Mo. To avoid contamination, all *Rhizobium* inoculated treatments were sown first. *Rhizobium* inoculation was done by using 10% sugar solution on seeds before sowing. The *Rhizobium* inoculants used were also obtained from AARI, Faisalabad.

Crop husbandry: Conventional tillage was adopted i.e., three times ploughing of field with tractor mounted plough followed by one planking for achievement of mandatory tilth. Mung bean cultivar AZRI-2006 was planted in spring 2015 and 201 at 30 cm apart rows and a plant to plant distance was maintained at 10 cm. Nitrogen (N, 23 kg ha^{-1}), phosphorus (P_2O_5, 57 kg ha^{-1}) and potash (K_2O, 62 kg ha^{-1}) were applied at recommended rates. 100% of P, K and one third of N were drilled at the time of sowing, while the remaining two third of N was top dressed in two equal splits at 25 days after sowing (DAS) and flowering = 55 DAS. Irrigations were applied at 25 DAS, flowering and pod formation stages.

Experimental layout and design: The experimental treatments consisted of 2 levels of *Rhizobium* inoculation (control and *Rhizobium*) and 5 levels of Mo (0, 3, 4, 5 and 6 g kg^{-1} seeds). The experimental design was randomized complete block design (RCBD) with 3 replica while arrangement was factorial. Dimension of each plot size was 5 m × 1.5 m containing 5 crop rows in each plot. The plots were interspaced by small terraces of 1 m to prevent contamination. The treatments were I_0= Not inoculated, I_1 (Inoculation), C_0 (0 g of Mo), C_1 (3 g of Mo), C_2 (4 g of Mo), C_3 (5 g of Mo), C_4 (6 g of), I_0C_0, I_1C_0, I_0C_1, I_1C_3, I_0C_2, I_1C_2, I_0C_3, I_1C_3, I_0C_4 and I_1C_4.

Plant harvesting and analysis: When plants got maturity, 2 middle rows in each plot was counted and harvested for calculation of grain yield. The border plants within each row were excluded. For yield components, 10 plants were sub-sampled from each plot to determine the plant height, pod bearing branches, pods per plant, pod length and number of seeds per pod. Number of nodules per plant, protein and chlorophyll, contents were recorded. Both pods were threshed manually. After that pods were dried upto 13% moisture content. Grain yield and 1000 grains weigh were calculated form each plot individually.

Chlorophyll and protein contents: Acetone (80%) extract of leaves was used for chlorophyll and carotenoids determination on spectrophotometer (663, 645 and 480 nm). The concentration of protein contents was determined by using bovine serum albumin as a standard (Hassan *et al*., 2017). Protein was determined at 520 nm wavelength on spectrophotometer.

Statistical analysis: The data from this experiment was analyzed using the software (STATISTIX 8.1) program. Analysis of variance (ANOVA) was applied to check the significance of amendments. However, Fisher's least significant difference was applied for comparison (p≤0.05) of treatment (Steel & Torrie, 1980).

Results

The plant height of mung bean observed with different treatments ranged between 47.2 and 59.4 cm during both years. Maximum increase (25%) in plant height over control was observed in Mo (4 kg/ha seed) + *Rhizobium phaseoli* from control. Highest plant height (59.4 cm) was statistically at par with 5 g Mo/kg of seed + *R. phaseoli* but significant over all other treatments. Minimum plant height (47.2 cm) was noted in control during both years (Table 1). The effects of different Mo levels and *R. phaseoli* were significant for pod bearing branches $plant^{-1}$ in both years. Plants received 4 kg/ha of seed + *R. phaseoli* gave higher (81%) no. of pod bearing braches $plant^{-1}$ over control (Table 1). Minimum pod bearing branches (6) were obtained in control during both years. Highest number of pods per plant (31) was observed from 4 g of Mo/kg seed + *R. phaseoli.* However, minimum number of pods per plant (25) was recorded in control (Table 1). When 4 g of Mo/kg seed + *R. phaseoli* was applied as seed treatment, maximum pod length (7.4 cm) was observed which was at par with 5 g of Mo/kg seed + *R. phaseoli*. Lowest pod length i.e., 5 cm was observed in control during both years (Table 1). The no. of seeds per pod of mung bean observed with different treatments ranged between 8-11. Maximum increase (43%) in no. of seeds per pod was observed over control in Mo (4 kg/ha seed) + *R. phaseoli*. The highest no. of seeds per pod (11) was statistically alike with 5 g Mo/kg seed + *R. phaseoli*. Minimum no. of seeds per pod (8) was noted in control during both years (Table 1).

Sole *Rhizobium phaseoli* inoculation and *Rhizobium phaseoli* + Mo had a significant effect on 1000-grain weight and seed yield compared to control (Table 2). In control, average 1000-grain weight was 56.8 g during both years. Highest 1000-grain weight (70.3 g) was found in 4 g Mo/kg seed + *R. phaseoli*. Maximum increase in 1000-grain weight (24%) was found in Mo/kg seed + *R. phaseoli* over control. Control had the lowest mean value 848.7 g for seed yield. Maximum increase (97%) in seed yield was observed in *R. phaseoli* + Mo 4 kg/ha seed over control.

Number of nodules plant^{-1} was significantly influenced by application of different levels of Mo and *Rhizobium phaseoli* during both years (Table 2). The lowest nodules number (3) was found in control. Addition of *R. phaseoli* + Mo 4 kg/ha seed gave maximum increase (109%) in number of nodules plant^{-1} over control. No significant change was observed in chlorophyll contents where Mo and *R. phaseoli* were applied (Table 2). However, protein contents of mung bean grains were improved due to addition of 4 g Mo/kg seed + *R. phaseoli* in both years (Table 2).

Discussion

Results of current experiment validated that *Rhizobium* inoculation is helpful in improving protein content and nitrogen fixation, yield and yield components of mung bean. Protein content were significantly higher where 4 g Mo/ kg seed + *Rhizobium* was applied as compared to control. The increased protein content was possibly due better nutrients uptake where Mo was applied with *Rhizobium* e.g. nitrogen and phosphorus availability which improves the overall quality parameters (Liu, 2002).

Our results inferred positive role of *Rhizobium* inoculation on mung bean which possibly due to symbiotic *Rhizobium* association with legumes. It is an established fact that the symbiosis of Rhizobium and legumes play an imperative role in enhancement of nodulation and yield in legumes (Ahmad *et al*., 2013). Fixation of atmospheric nitrogen significantly increase the bioavailability of N that is central part of mechanism for improvement in growth and yield of legumes associated with Rhizobium. Organic acid and roots exudates also played critical role for attraction of Rhizobium species in the rhizosphere. Better availability of Mo might also be involved in current study which is constituent of nitrogenase nitrate reductase enzymes synthesis and activity. For binding with diverse Apo proteins, nitrate reductase required Mo that act as a cofactor-Moco. Paudyal *et al*., (2007) also justified our argument while they reported higher number of nodules formation when incoulated with *Rhizobium* in the presence of Mo. Similarly, Hale *et al*., (2001) also reported a synergistic influence of Mo and nodule formation.

Application of Mo might play critical role in improvement of growth and grain yield of *V. radiata* i.e., plant height at maturity (25.38%), number of pod bearing branches plant^{-1} (80.71%), nodules plant^{-1} (109.33%), pods plant^{-1} (22.34%), pod length (49.40%), seeds pod^{-1} (43.32%), 1000- grain weight (23.65%), seed yield (96.91%). Our results are consistent with other workers like Bhuiyan *et al*., (2008) found that inoculation in the presence of P and Mo significantly enhanced plants height in mung bean comparative to uninoculated control. Togay *et al*., (2008) reported that both Mo and bacteria inoculation positively influence the number of pod bearing branches/ plant. Rabbani *et al*., (2005) argued that *Rhizobium* inoculation in the presence of P and Mo gave significantly better no. of pods/plant. Rahmani *et al*., (2008) also found similar results regarding *Rhizobium* and Mo combined application that significantly improve pod length. Landge *et al*., (2002) argued that inoculation of *Rhizobium* with P and Mo can enhance the seeds production per pod in mung bean. Rabbani *et al*., (2005) noted that *Rhizobium* with P and Mo have potential for significant improvement in 1000-seed weight. Significant improvement in biological yield was possibility due to Mo and seed inoculation that resulted in balanced growth pattern. Johansen *et al*., (2007) found that Mo seed treatments significantly enhanced the production of dry matter. Number of seeds per pod increased in mung bean when *Rhizobium* inoculant in association with P and Mo was applied (Landge *et al*., 2002). The increase in 1000-seed weight due to Mo and seed inoculation same results was observed by Rabbani *et al*., (2005) by the *Rhizobium* with P and Mo. An increase in seed yield with inoculation and Mo 4 g/kg seed was due to better plant growth development in yield contributing parameters. Deo and Kothari (2002) reported a significant improvement in yield with 3.5 g Sodium molybdate kg^{-1} was applied on seeds. Foliar application of Mo produced higher seeds per plant (Omer *et al.*, 2016).

Conclusion

In conclusion, molybdenum (Mo) applied in combination with *Rhizobium* significantly modified quality and yield attributes reported in this study. Better results were recorded in plots supplied with the rate of 4 g of Mo/kg seed. It was observed that co-application of Mo and *Rhizobium* improved protein contents of mung bean. Positive effects on number of nodules plant^{-1} elucidates the significance of *Rhizobium* and Mo on modification of nitrogen fixation that possibly play key role in enhancement of mung bean yield.

Table 1. Influence of different levels of molybdenum with and without *R. phaseoli* on mung bean growth attributes.

Treatment	Plant height (cm)		Pod bearing branches per plant		Pods per plant		Pod length (cm)		No. of seeds per pod	
A. Inoculation	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**	**2013**	**2014**
I_0	52.7 B	50.8 B	8.16 B	7.52 B	27.76 B	26.80 B	6.1 B	5.7 B	9.74 B	8.74 B
I_1	54.7 A	52.8 A	9.43 A	8.72 A	28.44 A	27.75 A	7.0 A	6.5 A	10.22 A	9.38 A
LSD	0.517	0.431	0.381		0.326	0.420	0.248	0.352	0.384	0.361
B. Conc. of Mo										
C_0	48.3 D	47.0 C	6.58 C	6.05 D	26.45 D	24.91 C	5.8 B	5.2 C	8.46 C	7.60 A
C_1	53.8 B	51.7 B	9.41 B	8.81 B	27.30 C	26.65 B	7.0 A	6.6 A	9.70 B	9.03 B
C_2	58.5 A	56.2 A	10.13 A	9.46 A	29.58 B	29.20 A	6.9 A	6.4 A	11.16 A	10.51 A
C_3	58.5 A	56.3 A	8.85 B	8.28 C	30.23 A	29.51 A	6.8 A	6.4 A	11.06 A	10.25 A
C_4	49.4 C	47.6 C	9.01 B	8.01 C	26.93 CD	26.10 B	6.2 B	5.8 B	9.53 B	7.91 C
LSD	0.818	0.682	0.602	0.406	0.515	0.664	0.393	0.556	0.608	0.571
C. Interaction										
I_0C_0	47.9 f	46.6 h	6.23 f	5.76 f	26.13 g	24.40 g	5.4 f	4.6 e	8.33 f	7.23 e
I_1C_0	48.7 f	47.4 h	6.93 f	6.33 f	26.76 efg	25.43 f	6.2 de	5.8 d	8.60 ef	7.96 cde
I_0C_1	52.8 d	50.7 f	8.76 cde	8.16 de	27.10 def	26.36 d-f	6.8 bc	6.2 bcd	9.26 de	8.60 c
I_1C_1	54.8 c	52.8 e	10.06 b	9.46 b	27.50 cd	26.93 cd	7.3 a	6.9 ab	10.13 bc	9.46 b
I_0C_2	56.4 b	54.1 d	9.10 cd	8.43 cd	28.06 c	27.73 c	6.0 de	5.7 d	10.93 ab	10.20 ab
I_1C_2	60.6 a	58.2 a	11.16 a	10.50 a	31.10 a	30.66 a	7.7 a	7.2 a	11.40 a	10.83 a
I_0C_3	57.5 b	55.6 c	8.13 e	7.66 e	30.06 b	29.06 b	6.5 cd	6.1 cd	10.93 ab	10.10 ab
I_1C_3	59.5 a	58.1 a	9.56 bc	8.90 bc	30.40 ab	29.96 ab	7.2 ab	6.8 abc	11.20 a	10.40 a
I_0C_4	48.9 ef	46.9 h	8.60 de	7.60 e	27.43 cde	26.43 de	5.8 ef	5.7 d	9.26 de	7.60 de
I_1C_4	49.9 e	48.4 g	9.43 bcd	8.43 cd	26.43 fg	25.76 ef	6.5 cd	6.0 cd	9.80 cd	8.23 cd
LSD	1.156	0.964	0.852	0.574	0.729	0.939	0.556	0.787	0.860	0.808

Values followed by dissimilar letters in the same column differ significantly at $p \leq 0.05$

Table 2. Influence of different levels of molybdenum with and without *R. phaseoli* on mung bean chlorophyll contents and yield attributes.

Treatment	Chlorophyll content (mg/g)		1000 grain weight (g)		No. of nodules per plant		Seed yield (g)		Protein contents (%)	
A. Inoculation	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
I_0	1.55 A	1.48 A	63.50 B	61.65 B	5.38 B	5.44 B	1309.7 B	1265.1 B	23.60 B	21.87 B
I_1	1.57 A	1.50 A	64 91 A	63.39 B	5.77 A	5.78 A	1424.3 A	1366.3 A	24.22 A	22.32 A
LSD	0.091	0.036	0.651	0.723	0.304		37.535	37.65	0.311	0.358
B. Conc. of Mo										
C_0	1.48 A	1.42 C	58.08 D	56.26 D	3.65 C	3.80 D	895.2 C	861.8 C	22.13 C	20.06 D
C_1	1.56 A	1.49 AB	63.68 B	62.40 B	6.10 AB	6.21 B	1403.3 B	1358.3 B	23.90 B	22.03 C
C_2	1.61 A	1.55 A	69.36 A	67.23 A	6.55 A	6.68 A	1575.8 A	1541.7 A	24.91 A	23.78 A
C_3	1.60 A	1.54 A	68.60 A	66.70 A	5.88 B	5.63 C	1597.5 A	1506.7 A	24.85 A	22.93 B
C_4	1.52 A	1.44 BC	61.31 C	60.01 C	5.72 B	5.71 C	1363.3 B	1310.0 B	23.76 B	21.66 C
LSD	0.144	0.057	1.030	1.143	0.481	0.425	59.349	59.541	0.491	0.566
C. Interaction										
I_0C_0	1.48 a	1.41 c	57.93 f	55.73 g	3.43 e	3.26 h	865.3 f	832.0 f	21.86 e	19.63 f
I_1C_0	1.49 a	1.43 bc	58.23 f	56.80 g	3.86 e	4.33 g	925 f	891.7 f	22.40 e	20.50 e
I_0C_1	1.55 a	1.48 abc	62.16 e	61.66 de	5.90 bc	5.70 de	1370 de	1326.7 de	23.73 cd	21.76 cd
I_1C_1	1.58 a	1.51 ab	65.20 d	63.13 d	6.30 b	6.73ab	1436.7 cd	1390.0 cd	24.06 bcd	22.30 bc
I_0C_2	1.61 a	1.54 a	67.46 c	65.20 c	6.00 bc	6.46 abc	1460 bc	1433.3 c	24.36 bc	23.76 a
I_1C_2	1.62 a	1.56 a	71.26 a	69.26 a	7.10 a	6.90 a	1691.7 a	1650.0 a	25.46 a	23.80 a
I_0C_3	1.60 a	1.55 a	68.26 bc	66.30 bc	6.43 ab	6.23 bcd	1526.7 b	1460.0 c	24.46 b	22.80 b
I_1C_3	1.61 a	1.54 a	68.93 b	67.10 b	5.34 cd	5.03 f	1668.3 a	1553.3 b	25.23 a	23.06 ab
I_0C_4	1.50 a	1.44 bc	61.70 e	59.36 f	5.17 d	5.53 ef	1326.7 e	1273.3 e	23.60 d	21.40 d
I_1C_4	1.54 a	1.45 bc	60.93 e	60.66 ef	6.26 b	5.90 cde	1400 cde	1346.7 de	23.93 bcd	21.93 cd
LSD	0.204	0.813	1.457	1.617	0.681	0.601	83.932	84.204	0.695	0.801

Values followed by dissimilar letters in the same column differ significantly at $p \leq 0.05$

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