

Effect of Molybdenum on leaf chlorophyll and biomass yield of Cowpea (*Vigna unguiculata*)

ABSTRACT

Molybdenum (Mo) is an essential micronutrient for plant. It plays an important key role in chlorophyll synthesis. The Experiment was conducted in Pot house at Department of Soil Science and Agricultural Chemistry, College of Agriculture, JNKVV, Jabalpur during *Kharif* season 2014. The soil of experimental site belongs to Kheri-series and taxonomically comes under the order Vertisols, sub order Sterts, great group Haplusterst, sub group TypicHaplusterst. In all total 14 number of treatment combinations were tried under factorial randomized block design replicated thrice. The result revealed that variations in observation were found with treatment of *Rhizobium* inoculation. Seed treatment of Mo @ 0.5 g ammonium molybdate kg^{-1} seed (StM₁) responded better, followed by soil application of Mo @ 1.0 kg Mo ha^{-1} soil (SaM₁) as main effects. With higher dose of Mo as seed treatment (StM₂) exerted significantly negative effect. Whereas higher dose of soil application of Mo (SaM₂) was marginally higher over no application of Mo which was seemed to be the buffer action of soil to nullify adverse effect of the micronutrient.

Keywords: Molybdenum, Cowpea, Legumes, Yield

Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) is an annual legume crop. Even though it is a minor pulse crop, it is important to the live food of millions of people as a vegetable. Cowpea seeds are a nutritious component in the human diet as well as a nutritious livestock feed. Cowpea constitutes 56-66% carbohydrate, 22-24% protein, 5.9-7.3% crude fibre. Molybdenum (Mo) is an essential micronutrient for plants. It plays an important key role in chlorophyll synthesis. In plants, it is absorbed as MoO_4^{2-} . Molybdenum deficient plants exhibit poor growth and low contents of chlorophyll, ascorbic acid, soluble sugar and shows reduced leaf blade formation, inter-veinal mottling and chlorosis around edges and tips of older leaves (Marschner, 2011, Liu, 2002), ultimately showing overall reductions in plant growth and development, expose the plant to susceptibility to pest damage, poor pod and/or grain development, low yield (Graham and Stangoulis, 2005).

Mo is necessary for the reduction of atmospheric nitrogen (N_2) to ammonia by nitrogenase. The symbiotic bacteria require about ten times more Mo for N_2 fixation than does the host plant (for protein synthesis). For this reason, Mo deficiency will commonly occur in legumes before it does in other plants, when grown in the same soil (Thibaund, 2005). Mo is also essential for nitrate reductase and nitrogenase enzyme activity (Westermann, 2005). The symbiotic bacterial enzyme nitrogenase is comprised of MoFe protein which is directly involved in the reduction of N_2 to NH_3 (Lambert *et al.*, 1998) during fixation process. 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).

Material and Methods

The Experiment was conducted in Pot house at Department of Soil Science and Agricultural Chemistry, College of Agriculture, JNKVV, Jabalpur during *Kharif* season 2014. The site is at 23° 12' 51.4" North latitude and 79° 56' 50.1" East longitudes at an altitude of 428 m above mean sea level. The soil of experimental site belongs to Kheri-series and taxonomically comes under the order Vertisols, sub order Sterts, great group Haplusterts, sub group TypicHaplusterts and family fine and montmorillonite-Hyperthermic. The pot house experiment on cowpea (cv. Ankurgomti) comprised supplementation of molybdenum (as ammonium molybdate) via two different modes of application viz., seed treatment (@ 0, 0.5, and 1.0 g Mo kg⁻¹ seed) and soil application (@ 0, 1.0, and 2.0 kg Ammonium molybdate ha⁻¹ soil). The seeds of cowpea were further treated with carrier lignite based Rhizobium (Jawahar Rhizobium @ 5.0 g kg⁻¹ seed). In all total 14 number of treatment combinations were tried under factorial randomized block design replicated thrice. The crop in pots received equally the basal application of fertilizer N : P₂O₅ : K₂O @ 20 : 50 : 70 kg ha⁻¹ in the form of urea, SSP and MOP. Equal number of pre-germinated seeds was sown in each pot and after establishment of seedlings; equal number of plants were maintained. The plants in each pot were drenched with sufficient quantity of water managed weed free condition (2 weedings). The harvested at mature stage. The soil parameters viz. Soil pH, Organic carbon (Walkley and Black (1934), Available nitrogen (Subbiah and Asija, 1956), Electrical conductivity, phosphorus (Olsen *et al.* 1954), potassium (Chapman and Pratt, 1961) and molybdenum (Ellis and Olson, 1950) were estimated by standard analytical methods. The data collected on pre harvest, post-harvest characters and chemical analyses were tabulated replication wise and analyzed by the method of analysis of variance. The treatment means were compared using least significant difference test at 0.05 level of significance described by Steel *et al.* (1997).

Results and discussion

Effect of seed treatment with molybdenum and *Rhizobium*

The results revealed that seed inoculation with molybdenum as well as Rhizobium and PSB containing biofertilizers exhibited superior performance over untreated control and the combined seed treatment with molybdenum and biofertilizers emerged as best and recorded the maximum leaf chlorophyll content, nodule number and dry weight and root dry weight (Table 2 and 3).

No. of Nodules per Plant

As a result, Rhizobium was found significant by 2.22 fold over the uninoculated plants. The data varied from 1.67 to 10.33 nodules No. plant⁻¹ at maturity with uninoculated control and Rh1xStM1xSaM1 and mean value 7.24. The main effect of seed treatment with Mo was also found significantly varying. StM1 responded significantly in increasing nodule numbers by 1.34 fold over untreated Mo plants. Seed treatment with Mo @ 1.0 g ammonium molybdate kg⁻¹ seed (StM2) exhibited no significant effect over that of Mo untreated plants. Soil application of Mo significantly increased No. of nodules plant⁻¹. Soil application of Mo application by @ 1 kg Mo ha⁻¹ (SaM1) increased nodule No. 1.26 fold over SaM0. The best interaction was Rh1xStM1 exhibiting 2.69 fold, followed by interaction of Rh1xStM0, Rh1xStM2 and Rh0xStM1 by 2.47, 2.33 and 1.54 folds increase, respectively over Rh0xStM0 (4.0 No. of nodules plant⁻¹). Mo supply in legumes increased molybdenum concentrations in nodules, improving N₂ fixation, development of seeds and other tissues. The found that Experiments with soybean and common bean have also shown that molybdenum fertilization

enhanced nitrogen-fixing symbiosis through increased nitrogenase activity rates and larger nodule formation (Parker and Harris, 1977; Adams, 1997; Vieira *et al.*, 1998).

Nodule biomass

The result showed that the main effect of Rhizobium was found significant by 2.22 fold over the un inoculated Plants. The highest value was recorded 11.33 nodule biomass plant⁻¹ at maturity with untreated control and Rh1xStM1xSaM and mean value of 28.92. The main effect of seed treatment with Mo was also found significantly varying. StM1 responded significantly in increasing nodule biomass by 1.21 fold over untreated Mo plants. Soil application of Mo significantly increased nodules biomass plant⁻¹. SaM1 was increased nodule dry weight 1.26 fold over SaM0. The interactions between RhxSaM were observed significantly higher over the lowest performing interaction Rh0 xSaM0. The best interaction was Rh1xSaM1 by 3.64 nodule biomass per plant.

Plant biomass weight

The result found that effect of Rhizobium was found significant by 35% over the uninoculated Plants. The effect of seed treatment with Mo was also found significantly varying. (StM1) responded significantly in increasing plant biomass by 12.69% over untreated Mo plants. Soil application of Mo significantly increased plant biomass plant⁻¹. SaM1 increased nodule biomass 8.09% over SaM0. The best interaction was Rh1xStM1 exhibiting 51.75%, followed by interaction of Rh1xStM0, Rh1xStM2 and Rh0xStM1 by 51.75, 39.47 and 34.21% increase, respectively over Rh0xStM0. The best interaction was Rh1xSaM1 by 42.83% followed by Rh1xSaM2 and Rh1xSaM0 by 37.93 and 34.48% increase plant biomass plant. Effect of all the interactions between RhxStMxSaM were significantly varied with the interaction of (Rh1xStM1xSaM1) increased plant biomass by Rh1xStM1xSaM1 by 80 % followed by the treatment interaction of Rh1xStM1xSaM0 and Rh1x StM0xSaM1 by 75 and 72 % increase, respectively over the control (Rh0xStM0xSaM0).

Microbial dynamic characteristics of soil

Rhizobium counts in rhizospheric soil

The results revealed that the Mo was had significant effects seed treatment. StM1 responded significantly in increasing rhizobial population in soil-1 by 1.23 fold over untreated Mo plants. StM2 exhibited no significant effect over that of Mo untreated plants. Soil application of Mo significantly increased rhizobial population plant⁻¹. SaM1 increased rhizobial population by 1.15 log fold over. The best interaction was Rh1xSaM1 by 1.38 fold followed by Rh1xSaM2 and Rh1xSaM0 by 1.22 and 1.07 log fold increase.

Microbial biomass carbon and nitrogen

The results found that the data varied from 70.40 to 98.53 at maturity plant⁻¹ with untreated control. An overall mean value of 85.24 at maturity. The main effect of Rhizobium was found significant by 13.38% over the uninoculated Plants (79.89 mg C kg⁻¹). The main effect of seed treatment with Mo was also found significantly varying. StM1 responded significantly in increasing microbial biomass carbon by 7.08% over untreated Mo plants (84.45 mg C g⁻¹). StM2 exhibited no significant effect over that of Mo untreated plants. Soil application of Mo significantly increased microbial biomass carbon plant⁻¹. SaM1 increased biomass C by 5.45% over SaM0. The best interaction was Rh1xStM1 exhibiting 20.05% followed by interaction of Rh1xStM0, Rh1xStM2 and Rh0xStM1 by 19.48 12.28 and 9.24% increase, respectively over Rh0xStM0 (79.56 mg C g⁻¹). The best interaction was Rh1xStM1 exhibiting 1.73 fold followed by interaction of Rh1xStM0, Rh1xStM2 and Rh0xStM1 by 1.50, 1.40 and 1.18 folds increase, respectively over Rh0xStM0.

Nutrient content (Mo and N) in plant and rhizospheric soil

The results revealed that the micronutrient and bio fertilizers treatments had significant effects on availability of major soil nutrients after harvest of cowpea; However, the changes in soil pH showed non- significant effect. Among the molybdenum and bio fertilizers seed treatment plots the maximum available nitrogen (211.92%),The main effect of Rhizobium was found significant by 25.52 % over the uninoculated Plants.Soil application of Mo significantly increased total nitrogen in plant. Soil application of Mo significantly increased total nitrogen. SaM1 increased total nitrogen in leaves by 6.47% over SaM0 (1.70% total nitrogen). The best interaction was Rh1xStM1 exhibiting 49.28% followed by interaction of Rh1xStM0, and Rh1xStM2 by 28.57and 32.85% increase, respectively over Rh0xStM0 (1.40% total nitrogen).Soil application of Mo significantly increased available nitrogen in soil. SaM1 increased available nitrogen by 3.76% over SaM0 (214.16 kg ha⁻¹ available nitrogen).The best interaction was Rh1xStM1 exhibiting 19.39% followed by interaction of Rh1xStM0, and Rh1xStM2 by 15.82 and 13.72 increase, respectively over Rh0xStM0. Molybdenum is an essential trace element and is vital for synthesis and activity of molybdoen-zymes such as nitrogen assimilation enzyme nitrate reductase and the nitrogen fixing enzyme nitrogenase, the key regulatory component for initiation of nodulation and maintenance of nitrogen fixation in legumes (Franco and Munns,1981).

Chlorophyll content in leaves

The result indicated that main effect of seed treatment with Mo was significantly varying. StM1 responded significantly in increasing total chlorophyll content by 18.39% over untreated Mo plants.The best interaction was Rh1xStM1 exhibiting 70.83% followed by interaction of Rh1xStM0, Rh1xStM2 and Rh0xStM1 by 50, 41.66 and 15.27% increase, respectively over Rh0xStM0.The best performing interaction was StM1xSaM1 by 40% followed by StM1xSaM0 and StM0xSaM1 by 32.5 and 28.75%, increase, total chlorophyll content.effect of all the interactions between RhxStMxSaM were significantly varied with the interaction of (Rh1xStM1xSaM1) increased total chlorophyll content in leaves by Rh1xStM1xSaM1 by 98.52% followed by the treatment interaction of Rh1xStM1xSaM0 and Rh1x StM0xSaM1 by 85.29 and 76.47%, increase, total chlorophyll content.

Yield of cropat harvest

The main results revealed that effect of Rhizobium was found that significant by 27.36% over the un inoculated Plants.StM1 responded significantly in increasing grain yield by 13.23% over untreated Mo plants.Soil application of Mo significantly increased grain yield plant-1. SaM1 increased grain yield by 7.61% over SaM0.the best interaction was Rh1xSaM1 by 37.15% followed by Rh1xSaM2 andRh1xSaM0 by 30.60 and 29.50%, increase grain yield. Effect of all the interactions between RhxStMxSaM were significantly varied with the interaction of (Rh1xStM1xSaM1) increased grain yield by Rh1xStM1xSaM1 by 75.33% followed by the treatment interaction of Rh1xStM1xSaM0 and Rh1x StM0xSaM1 by 72.66 and 70.66% increase, respectively over the control(Rh0xStM0xSaM0) stover yield per plant.Effect of all the interactions between RhxStMxSaM were significantly varied with the interaction of (Rh1xStM1xSaM1) increased stover yield plant-1 by Rh1xStM1xSaM1 by 42.63% followed by the treatment interaction of Rh1xStM1xSaM0 and Rh1x StM0xSaM1 by 38.37 and 36.04% increase, respectively over the control. Asokan *et al.* (2000) found that seed inoculation with bio fertilizers supplied the bioactive compounds such as vitamins, hormones and enzymes which influenced the plant metabolism activities. The availability and optimum supply of essential nutrients such as nitrogen and molybdenum favorably influenced the plant vigor, morphology

and metabolic processes, which ultimately enhanced the pods per plant and yield, increased of cowpea. Kothari (2002) stated that seed treatment of molybdenum can effectively supplement internal molybdenum deficiencies and rescue the activity of molybdo enzymes.

Conclusion

The experimental finding revealed that cowpea productivity under clay and sandy soil of humid zone can be enhanced through micronutrient management in the presence of biofertilizers. Adoption of seed treatment with molybdenum and biofertilizers along with recommended inorganic fertilizers would bring beneficial results for profitability in cowpea crop cultivation.

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Table 1. Initial soil characteristics of the experimental soil

Soil Parameter	Depth (0-15 cm)
Sand (%)	25.3
Silt (%)	17.9
Clay (%)	56.8
Soil pH	7.6
Electrical conductivity (dSm ⁻¹)	0.17
Organic carbon (gkg ⁻¹)	6.4
Available Nitrogen (kg ha ⁻¹)	197.56
Available Phosphorus (kg ha ⁻¹)	14.25
Available Potassium (kg ha ⁻¹)	293.66
Available Molybdenum (mgkg ⁻¹)	0.20
Rhizobial population (cfu)	1.3×10 ⁵
Biomass carbon (mgkg ⁻¹)	198
Biomass nitrogen (mgkg ⁻¹)	20.2

Table 2: Effect of micronutrients and bio fertilizers on growth and different characters of cowpea

Seed Mo	Soil Mo	No. of Nodule/Plant			Nodule biomass			Microbial biomass nitrogen			Rhizobial population			Total molybdenum		
		Rh0	Rh1	Mean	Rh0	Rh1	Mean	Rh0	Rh1	Mean	Rh0	Rh1	Mean	Rh0	Rh1	Mean
StM0	SaM0	1.67	8.67	5.17	1.67	8.67	5.17	23.57	30.20	26.89	3.744 (5.55x10 ³)	4.741 (5.51x10 ⁴)	4.243	0.34	0.44	0.39
	SM1	6.00	10.67	8.34	6.00	10.67	8.34	29.33	41.30	35.32	5.764 (5.50x10 ⁵)	6.797 (6.27x10 ⁶)	6.281	0.38	0.45	0.42
	SaM2	4.33	10.33	7.33	4.33	10.33	7.33	27.97	39.13	33.55	5.756 (5.70x10 ⁵)	6.716 (6.25x10 ⁶)	6.236	0.36	0.46	0.41
StM0.5	SaM0	6.67	11.00	8.84	6.67	11.00	8.84	29.87	43.80	36.84	5.726 (5.32x10 ⁵)	6.883 (7.64x10 ⁶)	6.305	0.40	0.49	0.45
	SaM1	8.33	11.33	9.83	8.33	11.33	9.83	30.53	45.57	38.05	6.402 (2.52x10 ⁶)	7.159 (1.44x10 ⁷)	6.781	0.41	0.51	0.46
	SaM2	3.50	10.00	6.75	3.50	10.00	6.75	26.43	37.63	32.03	5.416 (2.60x10 ⁵)	5.811 (6.48x10 ⁵)	5.614	0.38	0.52	0.45
StM1.0	SaM0	2.00	9.67	5.84	2.00	9.67	5.84	25.67	35.47	30.57	4.750 (5.62x10 ⁴)	5.785 (6.09x10 ⁵)	5.268	0.39	0.54	0.47
	SaM1	4.67	9.33	7.00	4.67	9.33	7.00	24.33	34.43	29.38	4.762 (5.78x10 ⁴)	5.782 (6.06x10 ⁵)	5.272	0.41	0.56	0.49
	SaM2	3.23	9.00	6.12	3.23	9.00	6.12	23.57	33.47	28.52	4.595 (3.93x10 ⁴)	4.728 (5.34x10 ⁴)	4.662	0.42	0.57	0.50
Mean		4.49	10.00	7.24	4.49	10.00	7.24	26.80	37.89	32.35	5.186	6.044	5.615	0.60	0.64	0.62

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Seed Mo	Soil Mo	Grain yield			Stover Yield			Total nitrogen			Available nitrogen			Total chlorophyll		
		Rh0	Rh1	Mean	Rh0	Rh1	Mean	Rh0	Rh1	Mean	Rh0	Rh1	Mean	Rh0	Rh1	Mean
StM0	SaM0	1.5	2.15	3.65	2.58	3.26	2.92	1.30	1.79	1.55	179.67	210.13	194.9	0.68	0.91	0.80
	SM1	2.1	2.56	4.66	3.22	3.51	3.37	1.61	2.09	1.85	201.87	225.53	213.7	0.85	1.20	1.03
	SaM2	1.97	2.46	4.43	3.21	3.47	3.34	1.58	2.01	1.80	199.2	224.63	211.91	0.82	1.14	0.98
StM0.5	SaM0	2.14	2.59	4.73	3.24	3.57	3.41	1.64	2.15	1.90	204.37	227.13	215.75	0.86	1.26	1.06
	SaM1	2.17	2.63	4.80	3.26	3.63	3.45	1.72	2.17	1.95	207.63	228.13	217.88	0.88	1.35	1.12
	SaM2	1.91	2.44	4.35	3.20	3.44	3.32	1.51	1.95	1.73	197.43	218.83	208.13	0.77	1.09	0.93
StM1.0	SaM0	1.85	2.36	4.21	3.17	3.36	3.27	1.45	1.89	1.67	192.41	216.8	204.60	0.73	1.08	0.91
	SaM1	1.74	2.34	4.08	3.11	3.34	3.23	1.40	1.85	1.63	191.53	216.87	204.20	0.72	1.01	0.87
	SaM2	1.69	2.28	3.97	3.07	3.30	3.19	1.36	1.83	1.60	188.47	211.93	200.20	0.71	0.96	0.84
Mean		1.9	2.42	4.32	3.12	3.43	3.27	2.10	2.22	2.16	195.84	219.99	207.92	0.80	1.11	0.95

Table 3: Interaction Effect of micronutrients and bio fertilizers on growth and different characters of cowpea

Rh x Sa	No. of Nodule/Plant			Nodule biomass			Microbial biomass nitrogen			Rhizobial population			Total molybdenum		
	Rh0	Rh1	Mean	Rh0	Rh1	Mean	Rh0	Rh1	Mean	Rh0	Rh1	Mean	Rh0	Rh1	Mean
SaM0	3.45	9.78	6.61	3.45	9.78	6.61	26.37	36.49	31.43	4.740 (5.49x10 ⁴)	5.803 (6.35x10 ⁵)	5.272	0.38	0.49	0.43
SaM1	6.33	10.44	8.39	6.33	10.44	8.39	28.06	40.43	34.25	5.643 (4.39x10 ⁶)	6.579 (3.80x10 ⁶)	6.111	0.40	0.51	0.45
SaM2	3.69	9.78	6.73	3.69	9.78	6.73	25.99	36.74	31.37	5.256 (1.80x10 ⁵)	5.752 (6.06x10 ⁵)	5.504	0.39	0.52	0.45
Mean	4.49	10.00	7.24	4.49	10.00	7.24	26.81	37.89	32.35	5.213	6.045	5.629	0.39	0.50	0.45

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Rh x Sa	Grain yield			Stover Yield			Total nitrogen			Available nitrogen			Total chlorophyll		
	Rh0	Rh1	Mean	Rh0	Rh1	Mean	Rh0	Rh1	Mean	Rh0	Rh1	Mean	Rh0	Rh1	Mean
SaM0	1.83	2.37	2.10	3.00	3.40	3.20	1.46	1.94	1.70	192.15	218.02	205.08	0.76	1.08	0.92
SaM1	2.00	2.51	2.26	3.20	3.49	3.35	1.58	2.04	1.81	200.34	223.51	211.92	0.82	1.19	1.00
SaM2	1.86	2.39	2.13	3.16	3.40	3.28	1.48	1.93	1.71	195.03	218.46	206.74	0.77	1.06	0.92
Mean	1.90	2.42	2.16	3.12	3.43	3.27	1.51	1.97	1.74	195.84	219.99	207.92	0.78	1.11	0.95

Table 4: Significant and non- significant different of micronutrients and bio fertilizers on growth and different characters of cowpea

SEm and CD5%	No. of Nodule/ Plant				Nodule biomass				Microbial biomass nitrogen				Rhizobial population				Total molybdenum			
	Rhizobium	StM	SaM	StMxSaM	Rhizobium	StM	SaM	StMxSaM	Rhizobium	StM	SaM	StMxSaM	Rhizobium	StM	SaM	StMxSaM	Rhizobium	StM	SaM	StMxSaM
Rhizobium	0.16	0.28	0.28	0.49	0.164	0.283	0.283	0.49	0.16	0.28	0.28	0.48	0.16	0.28	0.28	0.491	0.01	0.01	0.01	0.02
	0.47	0.81	0.81	1.41	0.469	0.812	1	1.40	0.46	0.79	0.79	1.38	0.46	0.81	0.81	1.407		0.00	0.00	
StM		0.20	0.35			0.2	0.34			0.20	0.34			0.2	0.34			0.01	0.01	
		0.58	1.00			0.575	0.99			0.56	0.97			0.57	0.99				0.00	
SaM			0.20				0.2				0.20				0.2				0.01	
			0.58				0.57				0.56				0.57		0.00	0.00	0.00	0.01

Conti.....

SEm and CD5%	Grain yield					Stover Yield				Total nitrogen				Available nitrogen				Total chlorophyll			
	Rhizobium	StM	SaM	PSBxP	StMxSaM	Rhizobium	StM	SaM	StMxSaM	Rhizobium	StM	SaM	StMxSaM	Rhizobium	StM	SaM	StMxSaM	Rhizobium	StM	SaM	StMxSaM
Rhizobium	0.057	0.099	0.099	SEm	0.171	0.06	0.10	0.10	0.17	0.00	0.01	0.01	0.00	0.26	0.44	0.44	0.77	0.00	0.00	0.00	0.01
	0.163	0.28	0.28	CD5%	490	0.16	0.28	0.28	490.00	0.01	0.01	0.02	0.01	0.74	1.28	1.28	2.21	0.01	0.01	0.01	0.02
StM		0.07	0.12				0.07	0.12		0.00	0.00		0.00		0.31	0.31			0.00	0.00	
		0.2	0.34				0.20	0.35		0.01	0.01		0.01		0.90	0.90			0.01	0.01	
SaM			0.07					0.07			0.00					0.31				0.00	
			0.2					0.20			0.01					0.90				0.01	

UNDER PEER REVIEW