



Synergistic effect of molybdenum and *Rhizobium* on growth and yield of vegetable cowpea (*Vigna unguiculata* (L.) Walp.)

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Abstract

A field experiment was conducted to investigate the interactive effects of molybdenum (Mo) application and *Rhizobium* inoculation on the growth, nodulation, yield attributes, and productivity of vegetable cowpea [*Vigna unguiculata* (L.) Walp.] cv. AVCP 1 under South Gujarat agro-climatic conditions. The study was laid out in a factorial randomized block design with twelve treatment combinations comprising six Mo levels (via soil application and seed treatment) and two *Rhizobium* levels (with and without seed inoculation). Results revealed that the combined application of Mo @ 300 g ha⁻¹ (soil) and *Rhizobium* inoculation (M₂R₁) significantly ($p < 0.05$) improved key growth and yield parameters, including early flowering (60.6 days), enhanced plant height (94.07 cm), highest number of pods plant⁻¹, nodules plant⁻¹ (155), and fresh and dry nodule weights. This treatment also recorded the highest total pod yield (5.97 t ha⁻¹) and marketable yield (5.86 t ha⁻¹), with a harvest index of 48.43%, compared to 3.83 t ha⁻¹ in the control (M₀R₀). The synergy between Mo and *Rhizobium* likely enhanced nitrogenase activity and nutrient assimilation, leading to improved vegetative growth and reproductive performance. The findings advocate for the integrated use of molybdenum (300 g ha⁻¹) and *Rhizobium* seed inoculation (10 mL kg⁻¹ seed) to optimize cowpea productivity sustainably, reduce reliance on synthetic nitrogen fertilizers, and support resource-efficient legume cultivation in molybdenum-deficient soils.

Keywords Fixation · Leguminous · Nitrogen and nodules

Introduction

Cowpea (*Vigna unguiculata* L. Walp.), commonly known as lobiya or chowla in India, is a vital leguminous vegetable crop belonging to *Fabaceae* family renowned for its adaptability to diverse agro-climatic conditions, particularly in arid and semi-arid regions. Its resilience to drought, ability to

thrive in low-fertility soils, and capacity for biological nitrogen fixation make it an indispensable component of sustainable agriculture systems. Beyond its agronomic advantages, cowpea serves as a significant source of income for smallholder and marginal farmers, contributing to both household food security and national economic growth.

Nutritionally, cowpea is a powerhouse, offering a rich profile of essential nutrients. Per 100 g of edible portion, it provides approximately 4.3 g of protein, 51 kilocalories, 0.09 mg of riboflavin, 941 IU of vitamin A, 14 mg of vitamin C, 80 mg of calcium, 8 g of carbohydrates, 2 g of dietary fiber, 0.2 g of fat, and 2.5 mg of iron (Singh 2014). This nutritional composition underscores its role in combatting malnutrition and enhancing dietary diversity, especially in regions where access to varied food sources is limited. India stands as the largest producer (25% of global production), consumer (27% of global consumption), and importer (14%) of pulses worldwide. Within the country, cowpea occupies a cultivation area of approximately 1.5 million hectares, yielding an annual production of 0.7 million tons and an average productivity of 4.6 tons per hectare. Notably,

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the state of Gujarat contributes significantly, with 0.31 lakh hectares under cowpea cultivation, producing 3.21 lakh tons annually and achieving a productivity of 10.55 tons per hectare (Anonymous 2020).

Despite its inherent advantages, cowpea production faces several constraints, including suboptimal soil fertility, limited availability of essential micronutrients, and inadequate biological nitrogen fixation. Among the micronutrients, molybdenum (Mo) plays a pivotal role in legume physiology. It is a critical component of the enzymes nitrogenase and nitrate reductase, which are essential for atmospheric nitrogen fixation and nitrate assimilation, respectively (Westermann 2005; Hansch and Mendel, 2009). Deficiencies in molybdenum can impair these enzymatic functions, leading to reduced nodulation, stunted growth, and diminished yields (Roychoudhury and Chakraborty 2022).

Rhizobium inoculation is another agronomic practice that enhances nitrogen fixation in legumes. The symbiotic relationship between Rhizobium bacteria and cowpea roots facilitates the conversion of atmospheric nitrogen into forms accessible to the plant, thereby improving growth and productivity. However, the efficiency of this symbiosis can be influenced by the availability of molybdenum, as it directly affects the activity of nitrogenase enzymes involved in nitrogen fixation (Rahman et al. 2008). While the individual roles of molybdenum supplementation and Rhizobium inoculation in enhancing cowpea productivity are well-documented, studies exploring their combined effects are limited. Understanding the synergistic impact of these inputs is crucial for developing integrated nutrient management strategies aimed at optimizing cowpea yield and quality.

Therefore, the present study was undertaken to evaluate the interactive effects of varying levels of molybdenum application and Rhizobium inoculation on the growth, nodulation, yield attributes, and nutrient uptake of vegetable cowpea cv. AVCP 1. The findings aim to inform best practices for enhancing cowpea productivity, particularly in regions characterized by molybdenum-deficient soils and limited access to synthetic fertilizers.

Materials and methods

The present investigation was conducted at the Vegetable Research Farm, Regional Horticultural Research Station, ASPEE College of Horticulture and Forestry, Navsari Agricultural University (NAU), Navsari, during the summer season. The experimental site is situated in the coastal region of South Gujarat at 20°37' N latitude, 72°54' E longitude, and 11.98 m above mean sea level. The climate of this region is tropical and falls under the South Gujarat Heavy Rainfall Zone (AES-III), receiving an average annual rainfall

of approximately 1500 mm, mostly concentrated between mid-June and the end of September. Meteorological data during the crop period, were recorded from the Agro-Meteorological Observatory at NAU. The experimental field comprised deep black cotton soil (Vertic Ustochrepts, Inceptisols). The experiment employed a factorial randomized block design (RBD) with three replications and 12 treatment combinations including six concentrations of molybdenum and two levels of *Rhizobium* i.e., R_0 : without *Rhizobium* seed treatment, R_1 : with *Rhizobium* seed treatment. The gross plot size was 3.15 m × 3.0 m, and the net plot size was 1.35 m × 2.4 m, with a spacing of 45 cm × 30 cm. Each plot contained 10 rows with 7 plants per row. The total experimental area measured was 435.1 m². The field was thoroughly prepared through ploughing and harrowing, followed by leveling with a wooden plank. Sowing was carried out manually, and gap filling was performed to ensure uniform plant population. A seed rate of 25 kg ha⁻¹ was used. Light irrigation was given immediately after sowing, and standard agronomic practices, including weeding, interculture, and plant protection measures, were followed as per the recommended package of practices. Manure and fertilizers were applied as per standard recommendations, with well-decomposed farmyard manure at 10 t ha⁻¹ and a basal dose of 20:40:00 kg ha⁻¹ NPK. The required quantity of manure was calculated based on plot size and uniformly incorporated during the final land preparation. Laboratory analyses related to the experiment were conducted in the Departments of Agricultural Chemistry and Biotechnology at NAU, Navsari.

The experiment's fixed effect consisted of two *Rhizobium* and six molybdenum treatments, with replications as random effects. The experimental data were analyzed using analysis of variance (ANOVA) for a factorial randomized block design (RBD) to test the significance of treatment effects. The standard errors were calculated using the pooled error term from the ANOVA table. Treatment means were compared using the Critical Difference (CD) at the 5% level of significance. Standard Error of Mean (S.Em.), Coefficient of Variation (C.V. %), and interactions between molybdenum (M) and Rhizobium (R) treatments were also computed to assess treatment variability and significance (Panse and Sukhatme 1985).

Results and discussion

Growth and yield attributes

The effect of combined molybdenum and *Rhizobium* application at rate of 300 g ha⁻¹ (M_2) and Rhizobium inoculation (R_1) was found significant ($p < 0.05$) for days to 50%

Table 1.1 Details of treatment used for the experiment

Treatment	Ammonium Molybdate (AM) (g ha ⁻¹)	AM per Plot / kg Seed (g plot ⁻¹ /g kg ⁻¹ seed)	Application Method
(A) Soil Application			
Mo: Control	—	—	No application
M ₁ : 200 g ha ⁻¹	368.0	0.35	AM dissolved in 1000 mL distilled water and sprayed uniformly on soil before sowing
M ₂ : 300 g ha ⁻¹	552.0	0.52	
(B) Seed Treatment			
M ₃ : 25 g ha ⁻¹	46.0	4.6	Seeds soaked in AM solution (100 mL distilled water) for 4 h before sowing
M ₄ : 50 g ha ⁻¹	92.0	9.2	
M ₅ : 6 mL AM Solution (12.5% Mo) kg ⁻¹ seeds	25.4	1.38	Seeds treated with 6 mL of 12.5% Mo solution per kg of seed

flowering, days to first picking, plant height, days to last picking (Table 1.2) and total number of pods plant⁻¹ (Table 1.3). The M₂R₁ treatment positively influenced various growth and nodulation parameters. It recorded the shortest duration to 50% flowering at 60.60 days, compared to 68.90 days in the control (MoRo). Plant height at final picking increased to 94.07 cm under M₂R₁, from 72.07 cm in the control. The number of primary branches per plant at final picking was highest in M₂R₁ at 7.53, while the control had 4.73. Notably, the number of nodules per plant at final picking reached 155.00 in M₂R₁, significantly higher than 47.33 in the control. These improvements can be attributed to molybdenum's role in enhancing nitrogenase activity and nodulation, thereby promoting vegetative growth and reproductive development (Li et al. 2023). It might also be credited due to combined seed treatment with molybdenum and seed inoculation with *Rhizobium* supplied the bioactive compounds such as vitamins, hormones and enzymes which influenced the plant metabolism (Siddiqui 2022). 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 growth of the plant. The similar findings recorded by Malik et al. (2003) and Movalia et al. (2018) in moong bean and Makoi et al. (2013) in common bean as well as Singh et al. (2007); Chatterjee and Bandyopadhyay (2017) showed that *Rhizobium* inoculation enhanced the growth parameters in cowpea. It might be due to combined application of seed treatment with molybdenum and *Rhizobium* which showed synergistic effect and resulted in improvement of morphological traits of cowpea. Chemical and biologically fixed nitrogen most dominated

Table 1.2 Effect of soil application of molybdenum and seed treatment of molybdenum as well as *Rhizobium* on days to 50% flowering, plant height (cm), days to first picking, last picking, primary branches plant⁻¹ and number of nodules plant⁻¹ at final picking of Cowpea cv. AVCP 1

Parameter	S.E.m. [±]	Days to 50% flowering		Plant height (cm) at final picking		Days to first picking		Days to last picking		Primary branches plant ⁻¹ at final picking		Number of nodules plant ⁻¹ at final picking	
		R ₀	R ₁	R ₀	R ₁	R ₀	R ₁	R ₀	R ₁	R ₀	R ₁	R ₀	R ₁
M ₀	68.90	67.93	72.07	75.13	75.40	74.80	87.74	89.92	4.73	5.43	47.33	119.33	
M ₁	66.73	62.50	84.00	85.47	71.27	70.00	90.73	110.36	5.07	7.00	80.67	260.00	
M ₂	67.00	60.60	86.87	94.07	72.73	69.20	94.24	112.10	6.53	7.53	125.33	155.00	
M ₃	69.40	71.53	74.53	81.07	76.20	74.73	90.85	91.34	4.93	5.53	98.67	108.00	
M ₄	68.00	70.10	81.00	82.67	77.33	75.93	92.34	95.74	6.33	6.97	101.00	127.00	
M ₅	70.46	62.60	80.60	86.87	74.93	74.07	93.98	106.90	6.80	7.20	82.67	228.00	

Table 1.3 Effect of soil application of molybdenum and seed treatment of molybdenum as well as *Rhizobium* on fresh weight of nodules plant⁻¹ (g), dry weight of nodules plant⁻¹ (g) at final picking, total number of pods plant⁻¹, pod length (cm), number of cluster plant⁻¹ and number of pods cluster⁻¹ of Cowpea cv. AVCP 1

Treatment	Fresh weight of nodules plant ⁻¹ (g)		Dry weight of nodules plant ⁻¹ (g) at final picking		Total number of pods plant ⁻¹	Pod length (cm)	Number of cluster plant ⁻¹	Number of pods cluster ⁻¹
	R ₀	R ₁	R ₀	R ₁				
M ₀	0.35	1.33	0.05	0.16	39.33	41.33	11.66	12.21
M ₁	1.24	2.30	0.11	0.73	46.67	49.67	11.91	12.69
M ₂	0.47	3.00	0.15	0.27	39.00	60.67	13.15	15.13
M ₃	1.17	0.75	0.10	0.14	39.67	45.00	11.92	12.63
M ₄	0.97	1.59	0.10	0.25	38.33	49.00	12.34	11.89
M ₅	0.77	1.40	0.13	0.25	41.00	49.33	12.17	14.57
Mean	0.83	1.73	0.11	0.30	40.67	49.17	12.19	13.19
M		R	M × R	M	R	M × R	M	R
S.E. \pm	0.08	0.04	0.11	0.02	0.01	0.02	1.32	0.76
C.D. 5%	0.22	0.12	0.22	0.04	0.02	0.06	3.86	2.23

factor influenced the vegetative growth characters because nitrogen on the internodes elongation by increasing the plasticity of cell wall through its effect on production of auxin formation in plant (Luo et al., 2020). Significantly ($p < 0.05$) higher value for number of nodules per plant, fresh and dry weight (g) of nodules per plant (Fig. 1.1) at final picking were obtained under the treatment combination of M₂R₂ and M₂R₃. That might be due to molybdenum supply in legumes increased concentrations in nodules size and numbers, improving nitrogen fixation, development of seeds and other tissues through increasing nitrogenase activity rates and larger nodule formation (Nadia and Kandil 2013). The same marked effect in terms of above-mentioned parameters have also been reported in common bean, soyabean and moong bean respectively by Kandil et al. (2013); Singh and Kumar (2008); Rahman et al. (2008), which showed that seed inoculation with *Rhizobium* significantly increased nodule number per plant as compared to control. Molybdenum and *Rhizobium* had non-significant influence on number of cluster plant⁻¹, pod length (cm) and number of pods cluster⁻¹ (Table 1.3) at final picking during the study. The combined application of molybdenum and *Rhizobium* inoculation (M₂R₁) markedly enhanced the growth and yield parameters of cowpea cv. AVCP 1. This treatment achieved the highest total pod yield (Fig. 1.2) of 5.97 t ha⁻¹, substantially surpassing the control (M₀R₀) yield of 3.83 t ha⁻¹. Similarly, the marketable pod yield per plot for M₂R₁ was 1.94 kg, compared to 1.25 kg in the control. The marketable pod yield per hectare (Fig. 1.3) also increased, with M₂R₁ yielding 5.86 t ha⁻¹ against the control's 3.75 t ha⁻¹. Furthermore, the harvest index for M₂R₁ was 48.43%, an improvement over the control's 40.09% (Table 1.3). The significantly higher value for pod yield (Fig. 1.3) was observed under the treatment combination of M₂R₁ due to the application of molybdenum and *Rhizobium* in combination significantly increased grain of legume and increased levels of available molybdenum and *Rhizobium* in the Rhizosphere might have positively affected the nodulation and vegetative growth of the plants which ultimately resulted in higher yields of both seed and biomass. The result confirmed by Rehman and Shah (2018), Nadia and Kandil (2013) in cowpea, Movalia et al. (2018); Bhuiyan et al. (2008) and Tahir et al. (2011) in moong bean as well as Yadav et al. (2017) in cluster bean.

Conclusion

The study suggests that soil application of molybdenum at 300 g ha⁻¹ along with seed treatment in 10 ml of *Rhizobium* culture kg⁻¹ seed is advisable to optimise pod yield of cowpea under South Gujarat agro-climatic conditions. Integrating molybdenum supplementation with *Rhizobium*

Fig. 1.1 Effect of molybdenum and Rhizobium application on nodules fresh weight (g plant^{-1}) and dry weight (g plant^{-1}) in cowpea

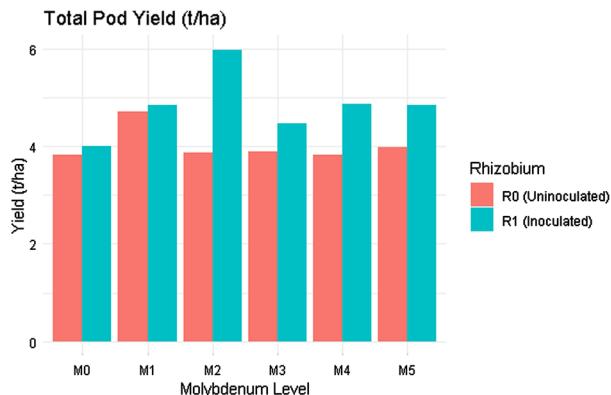
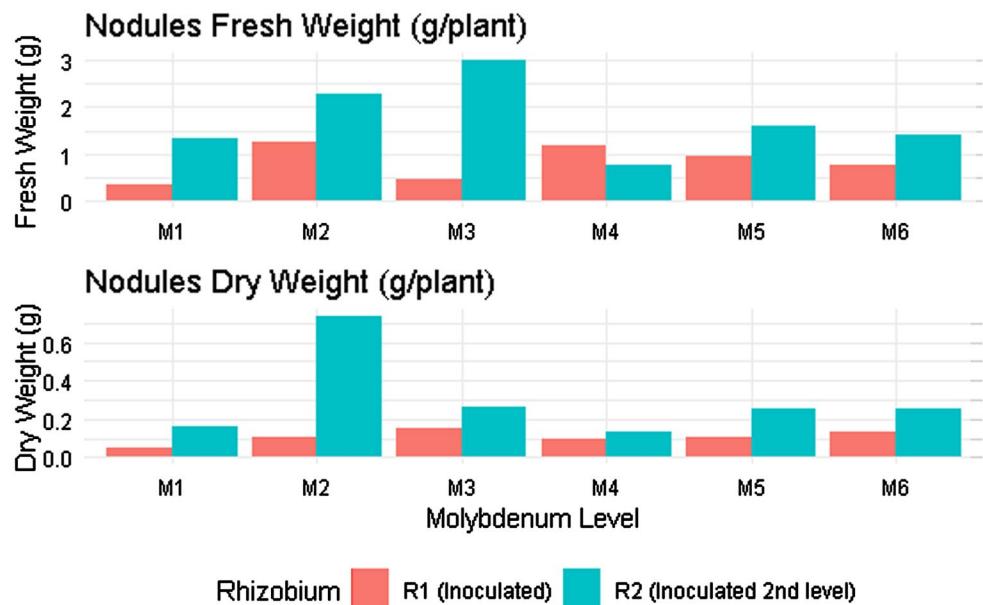
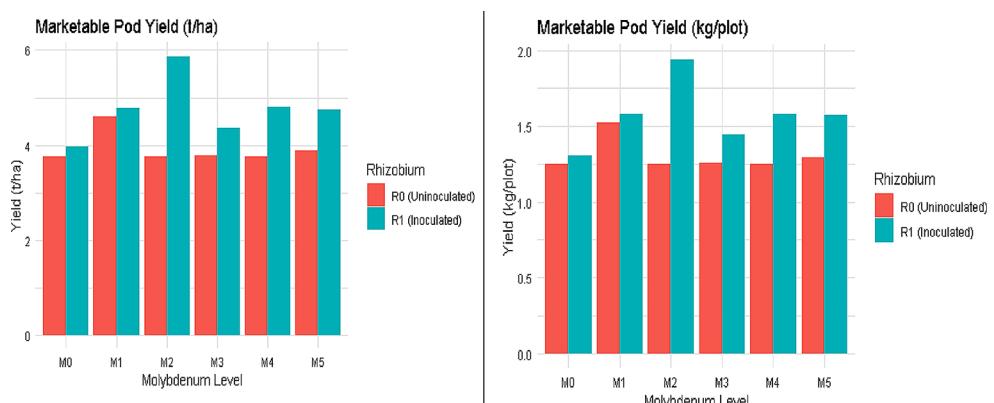


Fig. 1.2 Effect of molybdenum and Rhizobium application on total pod yield (t/ha) in cowpea

Fig. 1.3 Effect of molybdenum and Rhizobium application on marketable pod yield (t/ha) in cowpea
Effect of molybdenum and Rhizobium application on marketable pod yield (kg/plot) in cowpea



inoculation presents a sustainable strategy to enhance cowpea cultivation. Molybdenum is vital for the functionality of enzymes like nitrogenase and nitrate reductase, which are essential for nitrogen fixation and assimilation. When combined with Rhizobium inoculation, this synergy not only boosts nodulation and nitrogen assimilation but also fosters robust vegetative growth and reproductive development. Such an approach underscores the importance of micronutrient management in legume cultivation, ensuring optimal plant health and productivity. Moreover, adopting this integrated nutrient management practice aligns with sustainable agricultural principles by reducing dependency on synthetic nitrogen fertilizers, promoting environmental stewardship, and enhancing economic efficiency. Therefore, the combined application of molybdenum and Rhizobium

inoculation stands as a testament to the benefits of synergistic nutrient strategies in legume agriculture.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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