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Analyzing the Role of Different Molybdenum doses and *Rhizobium* Inoculation in Optimizing Nutrient Absorption and Yield in Vegetable Cowpea [*Vigna unguiculata* (L.) Walp.] cv. AVCP 1

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Authors' contributions

This work was carried out in collaboration among all authors. Author MK dissertation work, authors contributions, compilation of manuscript. Author DDC estimated yield parameters and compilation of manuscript. Authors AJ, RLM compilation of manuscript. Authors Sunil, HK compilation and communication of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Legumes, along with cereals, can be considered a main plant source of energy and quality proteins. A versatile legume crop, cowpeas offer economic, agronomic, environmental, and nutritional advantages. Cowpeas have a high total protein level and a relatively low fat content, making them nutritionally similar to other pulses. The study aimed to evaluate the effects of different rates of ammonium molybdate and *Rhizobium* inoculation on the yield and nutrient uptake of vegetable cowpea. There were 12 treatment combinations comprising six concentrations of molybdenum *i.e.* M_0:control, M_1: soil application of molybdenum @ 200 g ha^{-1}, M_2: soil application of molybdenum @ 300 g ha^{-1}, M_3: seed treatment of molybdenum 25 g ha^{-1} seeds, M_4: seed treatment of molybdenum 50 g ha^{-1} seeds and M_5: seed treatment of molybdenum 6 ml kg^{-1} seeds and two levels of *Rhizobium i.e*. R_0: without *Rhizobium* seed treatment, R_1: with *Rhizobium* (10 ml kg^{-1} seed) seed treatment in a Randomized Block Design (Factorial) with three replications.

Results indicated that the application of ammonium molybdate and *Rhizobium* inoculation significantly improved total cowpea yield. The highest yield was observed with the treatment combination of 300 g ha^{-1} ammonium molybdate (M_2) and *Rhizobium* inoculation (R_1). Nutrient analysis revealed that phosphorus, potassium and iron content in cowpea pods significantly increased with ammonium molybdate and *Rhizobium* treatments. The highest phosphorus and potassium contents were recorded with M_2 treatment, while the maximum iron content was found in M_3 treatment. *Rhizobium* treated seeds (R_1) also enhanced phosphorus, potassium and iron content in the pods. In terms of nutrient uptake, the highest nitrogen and phosphorus uptake were observed with the combined M_2R_1 treatment. Similarly, the highest potassium uptake in pods and total potassium uptake were recorded with M_2R_1 treatment, respectively. The interaction between ammonium molybdate and *Rhizobium* inoculation showed significant effects on nitrogen, phosphorus and potassium uptake, highlighting the importance of these treatments in enhancing cowpea nutrient absorption and yield. The use of biofertilizers can be highly successful in restoring agricultural soil, and this study highlights the possibility of combining Rhizobium inoculation with ammonium molybdate treatment to maximize nutrient uptake and enhance cowpea yield in sustainable agriculture production.

Keywords: Legumes; vegetable cowpea; cereals; molybdenum.

1. INTRODUCTION

Vegetables make up a major portion of the human diet in many parts of the world and play a significant role in human nutrition, especially as sources of phyto nutraceuticals: vitamins (C, A, B_1, B_6, B_9, E), minerals, dietary fiber and phytochemicals. Legumes form an integral group among vegetable crops. They have a low glycemic index (GI) in addition to being rich in nutrients. Legumes possess anti-cancer, anti-hyperlipidemic, anti-diabetic, anti-inflammatory, and anti-hypertensive qualities, and regular consumption helps control type 2 diabetes, cholesterol, and obesity [1]. By providing a substantial amount of N through N2 fixation, it increases soil fertility [2]. The cowpea is an essential crop among these legume vegetables, cultivated for its seeds and immature pods.

Cowpea (*Vignaunguiculata* (L.) Walp.) is one of the most important legume vegetable grown in India. The choice of cowpea as a vegetable is due to its palatability, nutritional factors and absence of metabolites or other toxins. The use of cowpea as a vegetable acts as a cheap source of protein (22-24%) in vegetarian dominated diets. Being rich in protein and containing many other nutrients, it is also known as vegetable meat. In India, cowpea is cultivated as one of the leading legume vegetable crop, covering an area of 23,012 ha, with an annual production of 1,33,587 tonnes and having productivity of 5800 kg ha^{-1}.

According to Yeswanth and Umesha (2023), molybdenum is a necessary element since it is a component of the nitrogenase enzyme, which is used by Rhizobium bacteria to fix nitrogen. In legume crops, molybdenum has a favorable impact on nodule formation, yield, and quality. Nitrate reduction, nodulation, nitrogen fixation, and overall metabolism are among the roles that molybdenum plays in leguminous plants [3]. Molybdenum was required for normal plant growth, reduction supply with molybdenum to the growth medium decreased activities of nitrate reductase and glutamine synthetase involved at initial steps of nitrate assimilation. The nitrogenase enzyme, used in nitrogen fixation, requires molybdenum for function in nitrogen-fixing bacteria. Nitrate reductase is a vital enzyme for nitrogen assimilation. A cofactor in this process is molybdenum [4]. Generally, Indian soils under natural condition are lacking in effective and specific strain of *Rhizobium*, which is responsible for symbiotic nitrogen fixation, growth stimulation of plant and enhances yield of pulses. *Rhizobium* inoculation also serves as a cheaper and usually more effective agronomic practice for ensuring adequate nitrogen nutrition of legumes than the application of nitrogen fertilizer. The purpose of the study was to observe the effect of molybdenum and bio fertilizers on growth parameters and yield attributes of cowpea. This can increase the production and productivity of the crop.

2. MATERIALS AND METHODS

The experiment conducted at vegetable research farm, Navsari Agricultural University, Navsari during summer season on cv. AVCP 1. There were 12 treatment combinations including six concentrations of molybdenum viz., soil application of M_1 200 g ha^{-1} and M_2 300 g ha^{-1}, seed treatment M_3 25, M_4 50 g ha^{-1} seeds and Ammonium molybdate solution M_5 (12.5 % Mo) before sowing with *Rhizobium i.e.,* R_0: without, R_1: with *Rhizobium* 10 ml kg^{-1} seeds inoculation. cowpea was planted with row to row distance of 45 cm and plant to plant distance of 30 cm in Factorial Randomized Block Design (FRBD) with three replication (Panse and Sukhatme 1985). All the recommended cultural practices and plant protection measures were followed. Observations were recorded from each plant of for yield and various qualitative characters and the data were subjected to statistical analyses. Methods followed for plant analysis: The pods and plants of cowpea were analyzed for N, P, K and Fe content following standard methods given in Table 1.The concentration of the nutrients was expressed in terms of percentage or mg kg^{-1} as the case may be. Subsequently, the uptake of these nutrients by pod and *haulm* portion of cowpea was also calculated by using the following formula.

Nutrient uptake by plant:

$$\text{N, P, K uptake}\left(\frac{\text{kg}}{\text{ha}}\right) = \frac{\text{Nutrient content (\%)x Yield}\left(\frac{\text{kg}}{\text{ha}}\right)}{100}$$

$$\text{Fe uptake (g/ha)} = \frac{\text{Nutrient content (mg/kg)x Yield(kg/ha)}}{1000}$$

3. RESULTS AND DISCUSSION

Yield attributes: The study revealed the effect of Ammonium molybdenum and *Rhizobium* was found significant in total yield (q) of cowpea. Maximum yield was observed in M_2 was 41.83 and *Rhizobium* (R_1) treated seed also help to enhance the cowpea yield was 48.28 and treatment combination M_2R_1 was found maximum in 59.67. This may be because to the presence of molybdenum and biofertilizers, such as *Rhizobium,* which increase root morphology through the production of cytokinins by *Rhizobium* and growth hormones like IAA. As a result, the plant would have been able to better absorb nutrients, producing more pods per plant and seeds per pod [8]. These findings are in collaboration with those reported by Kumar et al. [9] and Pragi et al. [10].

Nutrient Content and uptake by plant the Table 2 revealed that the phosphorus, potassium and iron content in pod increased significantly over control. The maximum phosphorus and potassium (0.26 and 1.78 %, respectively) contain was reported in 300 g ha^{-1} ammonium molybdate in soil application (M_2) while the iron contain was superior found in M_3 (197.57 mg kg^{-1}) whereas, the *Rhizobium* (R_1) treated seeds also helps to improve phosphorus, potassium and iron content in cowpea pod was (0.25%, 1.76% and 195.43 mg kg^{-1}, respectively). The rhizobacteria *Rhizobium* not only fixed nitrogen but also produced growth hormones, solubilized phosphates, and enhanced the growth and yield of non-legume plants by colonizing their roots [11]. By utilizing 1-aminocyclopropane-1-carboxylate (ACC), a plant ethylene precursor chemical, rhizobium may safeguard plants from ethylene stress. When plants are under stress, the enzyme ACC deaminase mediates the breakdown of ACC to ammonia and -ketobutyrate, which reduces the production of ethylene. ACC deaminase-producing rhizobium showed enhanced nitrogen fixation and nodulation traits [12].

The Table 3 showed the significant effect of nitrogen and phosphorus uptake in pod and haulm and total uptake of nitrogen and phosphorus in cowpea and the maximum result was reported in M_2 was 229.15, 200.91, 428.87, 17.57 and 21.01 kg ha^{-1}, respectively and while the total phosphorus uptake was found in M_4 (34.76) and the *Rhizobium* (R_1) treated seeds also helps to improve the uptake of nitrogen and phosphorus in pod and haulm and

Table 1. Methods employed for plant analysis

Nutrients	Method employed	Reference
N	Wet digestion (Chromic acid)	Trivedi *et al.* [5]
P and K	Wet digestion (diacid) followed by P: Spectrophotometric (Vanadomolybdophosphoric yellow colour method) K: Flame photometric	Jackson, [6]
Fe	Atomic Absorption Spectrophotometer (AAS)	Elwell and Gridley, [7]

Table 2. Effect of different molybdenum doses and Rhizobium on total pod yield (t ha^{-1}), phosphorus in pod (%), potassium in pod (%) and Fe content in pod (mg kg^{-1}) of cowpea cv. AVCP 1

	Total yield quintal per ha^{-1}			Phosphorus in pod (%)			Potassium in pod (%)			Fe content in pod (mg kg^{-1})		
	R_0	**R_1**	**Mean**	**R_0**	**R_1**	**Mean**	**R_0**	**R_1**	**Mean**	**R_0**	**R_1**	**Mean**
M_0	38.33	40.00	39.17	0.21	0.22	0.22	1.63	1.70	1.67	168.06	177.18	172.62
M_1	47.00	48.33	47.67	0.23	0.24	0.24	1.71	1.72	1.72	178.74	181.71	180.22
M_2	38.67	59.67	49.17	0.25	0.28	0.26	1.75	1.81	1.78	182.62	206.68	194.65
M_3	39.00	44.67	41.83	0.24	0.26	0.25	1.73	1.74	1.74	195.41	199.61	197.51
M_4	38.33	48.67	43.50	0.24	0.26	0.25	1.73	1.79	1.76	200.66	204.64	202.65
M_5	39.67	48.33	44.00	0.24	0.26	0.25	1.73	1.78	1.76	201.53	202.78	202.15
Mean	40.17	48.28		0.24	0.25		1.71	1.76		187.83	195.43	
	M	R	M × R	M	R	M × R	M	R	M × R	M	R	M × R
S.Em. ±	1.18	0.68	1.67	0.01	0.01	0.01	0.02	0.01	0.03	2.84	1.64	4.02
C.D. 5 %	3.45	1.99	4.88	0.03	0.01	NS	0.07	0.04	NS	8.34	4.81	NS
C.V. (%)	6.52			8.37			3.41			3.63		

**S.Em. ±: Standrad of Error of Mean, C.D. 5 %: Critical Difference and C.V. (%): Coefficient of Variation percentage*

Table 3. Effect of different molybdenum doses and *Rhizobium* on nitrogen uptake kg ha^{-1} in pod, nitrogen uptake kg ha^{-1} in haulm, total nitrogen kg ha^{-1}, phosphorus uptake kg ha^{-1} in pod, phosphorus uptake kg ha^{-1} in haulm and total phosphorus kg ha^{-1}of cowpea cv. AVCP 1

	Nitrogen uptake kg ha^{-1} in pod.			Nitrogen uptake kg ha^{-1} in *haulm*.			Total Nitrogen kg ha^{-1}			Phosphorus uptake kg ha^{-1}in pod			Phosphorus uptake kg ha^{-1} in haulm			Total phosphorus uptake kg ha^{-1} in		
	R_0	**R_1**	**Mean**	**R_0**	**R_1**	**Mean**	**R_0**	**R_1**	**Mean**	**R_0**	**R_1**	**Mean**	**R_0**	**R_1**	**Mean**	**R_0**	**R_1**	**Mean**
M_0	160.01	179.35	169.68	167.48	176.35	171.92	273.53	345.33	309.43	13.60	13.98	13.79	17.54	18.26	17.90	24.38	26.18	25.28
M_1	185.77	192.17	188.97	178.82	184.13	181.47	370.97	407.97	389.47	14.97	16.21	15.59	18.72	20.23	19.48	26.92	31.53	29.23
M_2	207.72	250.57	229.15	190.77	211.05	200.91	375.54	482.20	428.87	16.59	18.55	17.57	20.69	21.33	21.01	28.23	37.74	32.99
M_3	218.31	227.27	222.79	187.62	191.68	189.65	404.16	452.27	428.22	15.19	17.60	16.39	19.78	20.03	19.91	30.84	33.38	32.11
M_4	222.58	234.88	228.73	188.63	193.76	191.20	413.64	458.38	436.01	15.09	17.74	16.42	19.05	20.94	20.00	33.42	36.10	34.76
M_5	204.01	238.25	221.13	191.38	186.54	188.96	392.90	420.27	406.58	15.35	15.99	15.67	19.43	20.33	19.88	30.71	31.18	30.95
Mean	199.73	220.42		184.12	190.58		371.79	427.73		15.13	16.68		19.20	20.19		29.08	32.69	
	M	R	M ×R	M	R	M ×R	M	R	M ×R	M	R	M ×R	M	R	M ×R	M	R	M ×R
S.Em±	3.40	1.96	4.80	3.35	1.93	4.73	6.62	3.82	9.36	0.48	0.28	0.68	0.42	0.24	0.59	0.97	0.56	1.37
C.D.5%	9.96	5.75	14.09	9.82	5.67	NS	19.42	11.21	27.46	1.40	0.81	NS	1.23	0.71	NS	2.84	1.64	4.01
C.V..(%)	3.96			4.37			4.06			7.38			5.22			7.67		

**S.Em. ±: Standrad of Error of Mean, C.D. 5 %: Critical Difference and C.V. (%): Coefficient of Variation percentage*

Table 4. Effect of different molybdenum doses and *Rhizobium* on potassium uptake kg ha^{-1} in pod, potassium uptake kg ha^{-1} in haulm and total potassium kg ha^{-1} of cowpea cv. AVCP 1

	Potassium uptake kg ha^{-1} in pod			Potassium uptake kg ha^{-1} in haulm			Total potassium uptake kg ha^{-1}		
	R_0	**R_1**	**Mean**	**R_0**	**R_1**	**Mean**	**R_0**	**R_1**	**Mean**
M_0	83.95	90.10	87.03	50.66	56.56	53.61	127.37	145.31	136.34
M_1	91.12	97.16	94.14	67.83	68.50	68.17	168.25	151.47	159.86
M_2	98.03	116.63	107.33	70.63	74.96	72.80	180.44	182.80	181.62
M_3	93.07	109.39	101.23	60.87	68.27	64.57	151.65	165.85	158.75
M_4	110.17	113.98	112.08	59.19	70.31	64.75	174.50	180.85	177.67
M_5	108.62	109.72	109.17	68.81	69.58	69.20	175.86	176.11	175.99
Mean	97.49	106.16		63.00	68.03		163.01	167.07	
	M	R	M × R	M	R	M × R	M	R	M × R
S.Em. ±	1.87	1.08	2.65	1.18	0.68	1.66	2.03	1.18	2.88
C.D. 5 %	5.49	3.167	7.76	3.45	1.99	4.88	5.97	3.45	8.44
C.V. (%)	4.5			4.4			3.02		

**S.Em. ±: Standrad of Error of Mean, C.D. 5 %: Critical Difference and C.V. (%): Coefficient of Variation percentage* total nitrogen and phosphorus was 220.42, 190.58, 427.73, 16.68, 20.19 and 32.69 kg ha^{-1}, respectively. The interaction effect of ammonium molybdate and *Rhizobium* was found to be significant in nitrogen uptake in pod and total uptake of nitrogen and phosphorus in cowpea and the result reported the maximum in M_2R_1 was 250.57, 482.20 and 37.74 kg ha^{-1}, respectively. Table 4 shows the significant effect of potassium uptake in pod and haulm and total uptake of potassium. The maximum potassium uptake in pod was reported in M_4 (112.08 kg ha^{-1}) whereas the maximum potassium uptake in haulm and total uptake of potassium was found in M_2 was 72.80 and 181.62 kg ha^{-1} and the *Rhizobium* (R_1) treated seeds also helps to improve the potassium uptake in pod and haulm and total uptake of potassium was 106.16, 68.03 and 167.07 kg ha^{-1}. The interaction effect of ammonium molybdate and *Rhizobium* was found to be significant in potassium uptake in pod and haulm and total uptake of potassium in cowpea and the result reported maximum in M_2R_1 was 116.63, 74.96 and 182.80 kg ha^{-1}, respectively. Since molybdenum is a component of the enzyme nitrogenase, which is necessary for the symbiotic nitrogen fixation process, a notable increase in nutritional levels may be the result of increased nitrogen availability. These findings closely match those of Shivakumar and Kumutha [13] and Singh et al. [14]. Molybdenum increased the activity of the enzymes nitrogenase and nitrate reductase, which improved the soil's ability to fix nitrogen. This can be the result of elevated enzymatic activity in *Rhizobium* and plants. Similar findings were published by Amit et al. [15], who discovered that *Rhizobium* and molybdenum greatly enhanced the plant's uptake of nitrogen, phosphate and potassium in mung beans compared to the control. Additionally, Hossain *et al*. (2005) found that *Bradyrhizobium* and molybdenum applied together increased nitrogen uptake (132.2 kg ha^{-1}) in cv. G-2 (120.6 kg ha^{-1}). The use of micronutrients has been found to be beneficial in enhancing the uptake of nitrogen and phosphorus, while *Rhizobium* inoculation is known to enhance nodulation and nitrogen fixation in plants. This is because molybdenum replaces nitrogen fertilizers in legumes, enhances rhizobial infection, aids in the transport of sugars and there is a close relationship between the supply of micronutrients and nitrogen content [16]. According to Kyei-Boahen et al. [17], inoculants can enhance food safety by raising yield and nutritional value. The outcome supported the findings of Singh et al. [18] in cowpea and Jethra and Kothari [19] in fenugreek [20,21].

4. CONCLUSION

Rhizobium inoculation and ammonium molybdate application both significantly increase cowpea yield and nutrient uptake, according to the study's findings. *Rhizobium* seed inoculation with 300 g ha^{-1} ammonium molybdate produced the maximum yield, indicating that this combination successfully improves crop performance. Furthermore, with significant improvements in nutrient uptake, the treatments enhanced the nutrient content of cowpea pods, especially the levels of phosphorus, potassium, and iron. Their combined effect on the absorption of nitrogen, phosphorus, and potassium showed the synergy between ammonium molybdate and *Rhizobium* inoculation. The findings highlight the significance of using molybdenum and Rhizobium of this kind in terms to maximize nutrient uptake, boost crop yields, and encourage sustainable farming methods and improving soil health.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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