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EFFECT OF BIOFERTILIZERS (*RHIZOBIUM* AND PSB) WITH AND WITHOUT MOLYBDENUM ON BIOMASS PRODUCTION, UPTAKE AND QUALITY OF CHICKPEA (*CICER ARIETINUM* L.)

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ABSTRACT

The use of effective bio-inoculants in chickpea cultivation is a potential strategy to improve biomass yield and nutrient absorption in rainfed conditions. This research assessed the effects of biofertilizers, namely *Rhizobium* and phosphate-solubilizing bacteria (PSB), in conjunction with molybdenum on nutrient absorption and quality metrics of chickpea (*Cicer arietinum* L.). Field experiment was executed during the rabi season of 2020-21 at the Research Farm, Krishi Vigyan Kendra, JNKVV, Jabalpur. The trial had a randomized block design including 10 treatments, each replicated three times. The treatments included different combinations of *Rhizobium* (10 g, 15 g, and 20 g), PSB (20 g), and ammonium molybdate (0.5 g and 1 g) per kilogram of seed, alongside a control group for comparison. The findings indicated that the treatment including *Rhizobium,* PSB, and molybdenum (1g kg^{-1} seed) yielded the most substantial enhancements in biomass production and nutrient absorption. Nitrogen intake was 61.29 kg ha^{-1} in seeds and 26.20 kg ha^{-1} in stover, while phosphorus uptake was 6.68 kg ha^{-1} in seeds and 8.77 kg ha^{-1} in stover. This treatment resulted in the greatest protein concentration in chickpea seed, reaching a maximum of 22.86%. Moreover, the integration of biofertilizers and molybdenum enhanced Sulphur absorption in both seeds (2.77 kg ha^{-1}) and stover (4.73 kg ha^{-1}), surpassing treatments devoid of molybdenum. The results indicate that using biofertilizers in combination with molybdenum offers a sustainable approach to improving the nutritional content and yield of chickpea in rainfed environments, especially on Vertisol soils.
***Keywords* :** Molybdenum, *Rhizobium,* PSB, Chickpea

Introduction

India is the leading producer of chickpea, contributing 64% to world output. Pulses have significant importance in the Indian diet since they serve as the primary source of protein for the mostly vegetarian population. Chickpeas include 13-33% protein, 40-55% carbohydrates, and 4-10% oil. It comprises 50% oleic acid and 40% linoleic acid. It is a superior source of vitamins B6, C, and zinc. The growing global population poses a significant challenge for achieving food and nutritional security during the present climate change crisis (FAO, 2022). Moreover, the deterioration of soil health over time presents an additional barrier to attaining increased agricultural output (Aloo *et. al.* 2022). Consequently, it is unavoidable that the food requirements of the growing population would be satisfied by enhanced agricultural production (Phyton 2024). The disproportionate use of chemical fertilizers, especially nitrogenous fertilizers, enhances crop yield but disrupts ecological equilibrium, compromising soil, water, and human health (Saini *et al.*, 2021; Uddin *et al.*, 2023). Several leguminous plants may form nitrogen-fixing root nodules with the assistance of microbes known as rhizobia (Sprent, 2001). This connection is crucial in biological nitrogen fixing. The efficacy of this process is contingent upon the morphological and physiological properties of the host legume plant, which facilitate nitrogen uptake by these bacteria (Cieslarova *et al.,* 2012). Legumes require sufficient quantities of essential micronutrients or biofertilizers for optimal growth and development (Akter *et al.,* 2020). *Rhizobium* is crucial for enhancing soil health by facilitating plant growth and development via root nodule formation and nitrogen fixation (Tena *et al*., 2016; Pulido-Suárez *et al.,* 2021). Khiangte *et al.* (2023). *Rhizobium* inoculants enhance the absorption of NPK and other plant nutrients by crops, stimulating plant growth and development, which leads to enhanced agricultural yields (Singh and Gupta, 2018; Basu *et al.,* 2021). Numerous studies have shown that the production of various crops may rise by around 25% owing to the effects of *Rhizobium*, whereas the use of nitrogen- and phosphorus-based fertilizers can diminish the yield by approximately 25% to 50%, respectively (Khan and Chattopadhyay 2009; Sabez *et al.* 2012). Research indicates that using efficient Rhizobia strains may improve bean production (Tena *et al*., 2016, Bhuiyan *et al*., 2008). Phosphorus (P) is the second most important macronutrient, with mineral P fertilizers and manures being used as primary supplies of P for agricultural crops. Phosphorus shortage is regarded as a primary obstacle to agricultural output, impacting soil fertility and productivity globally (Zhang *et al*., 2019). The use of phosphatic fertilizers is a prevalent method to sustain and enhance the phosphorus status of soils (Da Costa *et al.,* 2015). Nonetheless, the persistent use of artificial fertilizers has resulted in several environmental problems globally (Biswakarma *et al.,* 2018). Therefore, it is essential to investigate environmentally sustainable methods to enrich the soil while preserving the integrity of agricultural systems and the quality of the environment (Kumar *et al*., 2019). The use of plant growth-promoting microorganisms is considered a viable strategy to mitigate the adverse effects of chemical fertilizers on the environment. The use of microorganisms to enhance plants' capacity to absorb nutrients from the soil is gaining widespread acceptance (Qiu *et al.,* 2019). The capacity of microorganisms to solubilize phosphate is increasingly used to convert phosphorus from inaccessible to easily available forms for plants (Bargaz *et al.,* 2018). These advantageous bacteria assist plants by improving phosphorus availability and absorption from phosphorus-deficient soils, demonstrating efficacy in promoting farm sustainability and reducing chemical fertilizer consumption to some degree (Robinson *et al.,* 2016; Wu *et al.,* 2019). The phosphorus-solubilizing bacteria (PSB) solubilize phosphorus by secreting organic acids and enzymes, hence facilitating its availability for plant absorption (Singh *et al*., 2013; Prabhu *et al*., 2019). Consequently, advantageous microorganisms have significant potential to sustain and enhance the quality and fertility of soils (Paredes and Lebeis, 2016). Molybdenum (Mo) is a crucial trace micronutrient, integral to over 60 enzymes that facilitate diverse redox processes (Baker and Philbeam, 2007). The enzymes nitrogenase and nitrate reductase play a pivotal role in nitrogen fixation, hence influencing nitrogen transport in plants (Liu and Yang, 2001). The lack of molybdenum in crops results in diminished flower development, reduced size, and insufficient maturity, ultimately leading to decreased grain production. Mo seed priming enhanced grain production and net return in chickpeas (Singh *et al*., 2014). The incorporation of an adequate quantity of molybdenum with *Rhizobium* inoculant is essential and has surfaced as a novel approach for promoting growth, enhancing yield, and maintaining soil fertility (Murgese *et al*., 2020; Fasusi *et al*., 2021). It facilitates nitrogen acclimatization and the generation of phytohormones (Rana *et al*., 2020). The application of Mo enhances canopy development, nodulation, and crop production (Khan *et al.,* 2019). This research aimed to determine whether the application of a combination of *Rhizobium*, PSB and Molybdenum improves the uptake of nutrients and quality in chickpea.

Material and Method

Study site and plant materials

Field experiments were conducted during the rabi season of 2020-21 at the Research Farm of Krishi Vigyan Kendra JNKVV in Jabalpur to study the effect of molybdenum in combination with *Rhizobium* and PSB on symbiotic traits and grain yield of crops cultivated under rainfed conditions in Vertisol with a pH of 7.5, electrical conductivity of 0.198 dSm^{-1}, and available nitrogen, phosphorus, and potassium levels of 221.3, 13.15, and 286.6 kg ha^{-1}, respectively. The cultivar was JG-14.

Treatment Details

The recommended doses of P (60 kg ha^{-1}) and K (20 kg ha^{-1}) were applied as basal through DAP and MOP at the time of sowing. The experiment was laid out in a randomized block design with 10 treatments replicated thrice. The control treatment was designated as T1. Treatment T2 consisted of 10 g of *Rhizobium* and 20 g of phosphate-solubilizing bacteria (PSB) per kilogram of seed. Treatment T3 included 15 g of *Rhizobium* and 20 g of PSB per kilogram of seed. Treatment T4 applied 20 g of *Rhizobium* and 20 g of

PSB per kilogram of seed. Treatment T5 included 10 g of *Rhizobium*, 20 g of PSB, and 0.5 g of ammonium molybdate (AM) per kilogram of seed. Treatments T6 and T7 incorporated 15 g and 20 g of *Rhizobium*, respectively, along with 20 g of PSB and 0.5 g of AM per kilogram of seed. Treatments T8, T9, and T10 repeated the *Rhizobium* and PSB combinations of 10 g, 15 g, and 20 g per kilogram of seed, respectively, but with an increased AM dose of 1 g per kilogram of seed.

Observations

At 30 and 60 days after sowing, as well as at harvest, five plants from each treatment were carefully uprooted and dried individually in the shade for 24 hours. Subsequently, they were placed in a hot air oven at 65°C for 72 hours, after which the final weight (g) was recorded for each treatment at the specified intervals. The average weight was determined by weighing a total of five plants. The plants were harvested by hand at the stage of physiological maturity, and samples of both grain and stover were collected for subsequent analysis. A mechanical grinder was employed to grind oven-dried plant samples to a fine powder. On an electric hot plate, the grounded samples of grain and stover, each weighing 1.0 g, underwent digestion with a di-acid mixture consisting of HNO_3 and $HClO_4$ in a 3:1 ratio (Kumar and Dhaliwal 2021). The nitrogen content in the plant was assessed through the micro Kjeldahl digestion and distillation method (Amma, 1989) utilizing the KEL PLUS (Pelican equipment's) system. The phosphorus content in the plant extract was assessed using the Vanado-molybdo phosphoric acid yellow colour method, employing a blue filter (Koenig and Johnson, 1942). The determination of potassium in the plant was conducted utilizing a flame photometer, following the methodology outlined by Black (1965). The determination of sulphur was conducted using the Turbidimetric method with a Spectronic 20 at a wavelength of 420 nm (Tandon, 1995). Nutrient uptake by chickpea was calculated in kg^{-1} in relation to (dry matter production) yield ha^{-1} by using the following –

Nutrient uptake (kg ha^{-1}) = Nutrient content (%) X yield (kg ha^{-1}) / 100

Statistical Analysis

The recorded data were analyzed with the 'Analysis of Variance (ANOVA)' approach as outlined by Gomez and Gomez (1984).

Result and Discussion

Biomass production per plant

Biomass production increased in all treatments compared to the control at 30 DAS, 60 DAS, and at harvest. At 30 days after sowing (DAS), the maximum biomass was recorded in T10 (20 g *Rhizobium* + 20 g PSB + 1 g AM) at 2.81 g, compared to 1.27 g in the control group. At 60 days after sowing, T10 exhibited the highest biomass at 9.81 g, surpassing the control group, which recorded 7.27 g (Table 1 and Figure 1). At harvest, T10 exhibited the highest biomass at 28.81 g, whereas the control yielded the lowest at 24.27 g. The notable increase in plant biomass observed under treatment T7 (20g *Rhizobium* + 20g PSB + AM - 0.5 g kg^{-1} seed) can be attributed to the enhanced microbial activity facilitated by the inoculated rhizobia and the presence of molybdenum, which collectively promote greater biomass accumulation per plant. This can be attributed to the role of molybdenum as a crucial component of nitrogenase and nitrate reductase enzymes, which are necessary for biological nitrogen fixation and nitrogen transformation within the plant system. Similar results were reported by Valenciano *et al.* (2011), Raddy and Swamy (2000), and Khan and Khan (2010).

Table 1 : Biomass production per plant at different growth stages of Chickpea

Treatments	Biomass production (g)		
	30 DAS	**60 DAS**	**At harvest**
T_1 - Control	1.27	7.27	24.27
T_2 - 10 g *Rhizobium* + 20g PSB kg^{-1} seed	2.61	9.61	28.61
T_3 - 15 g *Rhizobium* + 20g PSB kg^{-1} seed	2.67	9.67	28.67
T_4 - 20 g *Rhizobium* + 20g PSB kg^{-1} seed	2.73	9.73	28.73
T_5 - 10g *Rhizobium* +20g PSB kg^{-1} seed + AM (0.5g kg^{-1} seed)	2.63	9.63	28.63
T_6 - 15g *Rhizobium* + 20g PSB kg^{-1} seed + AM (0.5g kg^{-1} seed)	2.69	9.69	28.69
T_7 - 20g *Rhizobium* + 20g PSB kg^{-1}seed + AM (0.5g kg^{-1} seed)	2.77	9.77	28.77
T_8 - 10g *Rhizobium* + 20g PSB kg^{-1} seed + AM (1g kg^{-1} seed)	2.65	9.65	28.65
T_9 - 15g *Rhizobium* + 20g PSB kg^{-1} seed + AM (1g kg^{-1} seed)	2.71	9.71	28.71
T_{10} - 20g *Rhizobium* + 20g PSB kg^{-1} seed + AM (1g kg^{-1} seed)	2.81	9.81	28.81
SEm±	0.04	0.15	0.37
CD at 5%	0.12	0.46	1.11

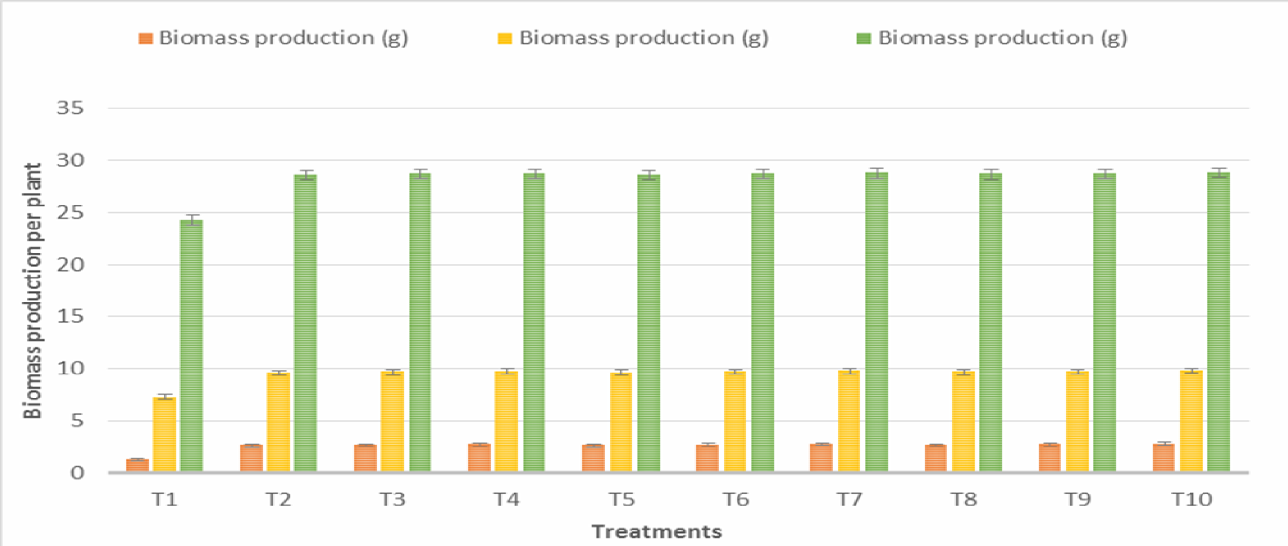

Fig. 1: Biomass production per plant at different growth stages of Chickpea

Major nutrient uptake (N, P, K and S) by seed and stover

The highest absorption of nitrogen, phosphorus, and Sulphur by seed and stover was observed with treatment T10, which comprised 20g of *Rhizobium*, 20g of PSB per kg of seed, and 1g of AM per kg of seed. Treatment T7, consisting of 20g of *Rhizobium*, 20g of PSB per kg of seed, and 0.5g of AM per kg of seed, exhibited statistically comparable results to T10. This effect may be attributed to the enhanced microbial activity resulting from the application of bio-fertilizers and the enhanced nitrogen fixation facilitated by increased nitrogenase and nitrate reductase activities, when combined with molybdenum application with *Rhizobium* and PSB. The results indicated that treatment T7 (20g *Rhizobium* + 20g PSB kg^{-1} seed + AM -0.5g kg^{-1} seed) had a significant impact on potassium uptake in seeds, followed by T10 (20g *Rhizobium* + 20g PSB kg^{-1} seed + AM -1g kg^{-1} seed) and T9 (15g *Rhizobium* + 20g PSB kg^{-1} seed + AM-1g kg^{-1} seed), which recorded the values of 9.76 and 9.54 kg ha^{-1}, respectively (Table 2 and Figure 2). No conclusive response in potassium uptake by stover under different treatments was seen. The disparate absorption of N, P, K, and S across diverse treatments may result from differences in nodulation, growth, and yield influenced by the addition of molybdenum with *Rhizobium* and PSB compared to the control group. The advantageous impact of molybdenum and bio-fertilizer treatment on chickpeas and other legumes has been reported by Gupta and Gangwar (2012), Sapatnekar *et al.* (2002), Thenua and Sharma (2011), and Gupta and Sahu (2012).

Table 2: Nutrient uptake (kg ha^{-1})

Treatments	N uptake (Kg ha^{-1})		P uptake (Kg ha^{-1})		K uptake (Kg ha^{-1})		S uptake (Kg ha^{-1})	
	Seed	**Stover**	**Seed**	**Stover**	**Seed**	**Stover**	**Seed**	**Stover**
T_1 - Control	36.19	12.86	4.21	5.66	6.75	31.95	1.93	4.34
T_2 - 10 g *Rhizobium* + 20g PSB kg^{-1} seed	49.43	16.64	5.38	7.37	8.57	33.60	2.36	4.38
T_3 - 15 g *Rhizobium* + 20g PSB kg^{-1} seed	53.84	20.79	5.83	7.46	9.09	33.32	2.48	4.39
T_4 - 20 g *Rhizobium* + 20g PSB kg^{-1} seed	58.75	23.70	6.37	8.56	9.36	34.50	2.68	4.61
T_5 - 10g *Rhizobium* +20g PSB kg^{-1} seed + AM (0.5g kg^{-1} seed)	50.33	18.34	5.47	7.25	8.67	32.68	2.38	4.41
T_6 - 15g *Rhizobium* + 20g PSB kg^{-1} seed + AM (0.5g kg^{-1} seed)	56.18	21.63	6.09	7.57	9.10	33.80	2.57	4.51
T_7 - 20g *Rhizobium* + 20g PSB kg^{-1}seed +AM (0.5g kg^{-1} seed)	59.89	24.90	6.51	8.68	9.94	34.68	2.73	4.66
T_8 - 10g *Rhizobium* + 20g PSB kg^{-1} seed + AM (1g kg^{-1} seed)	51.08	19.42	5.62	7.32	8.90	32.86	2.42	4.44
T_9 - 15g *Rhizobium* + 20g PSB kg^{-1} seed + AM (1g kg^{-1} seed)	57.31	22.35	6.19	7.81	9.54	34.06	2.61	4.60
T_{10} - 20g *Rhizobium* + 20g PSB kg^{-1} seed + AM (1g kg^{-1} seed)	61.29	26.20	6.68	8.77	9.76	34.89	2.77	4.73
SEm±	1.06	0.38	0.12	0.18	0.15	0.65	0.06	0.08
CD at 5%	3.16	1.12	0.36	0.54	0.45	NS	0.18	0.25

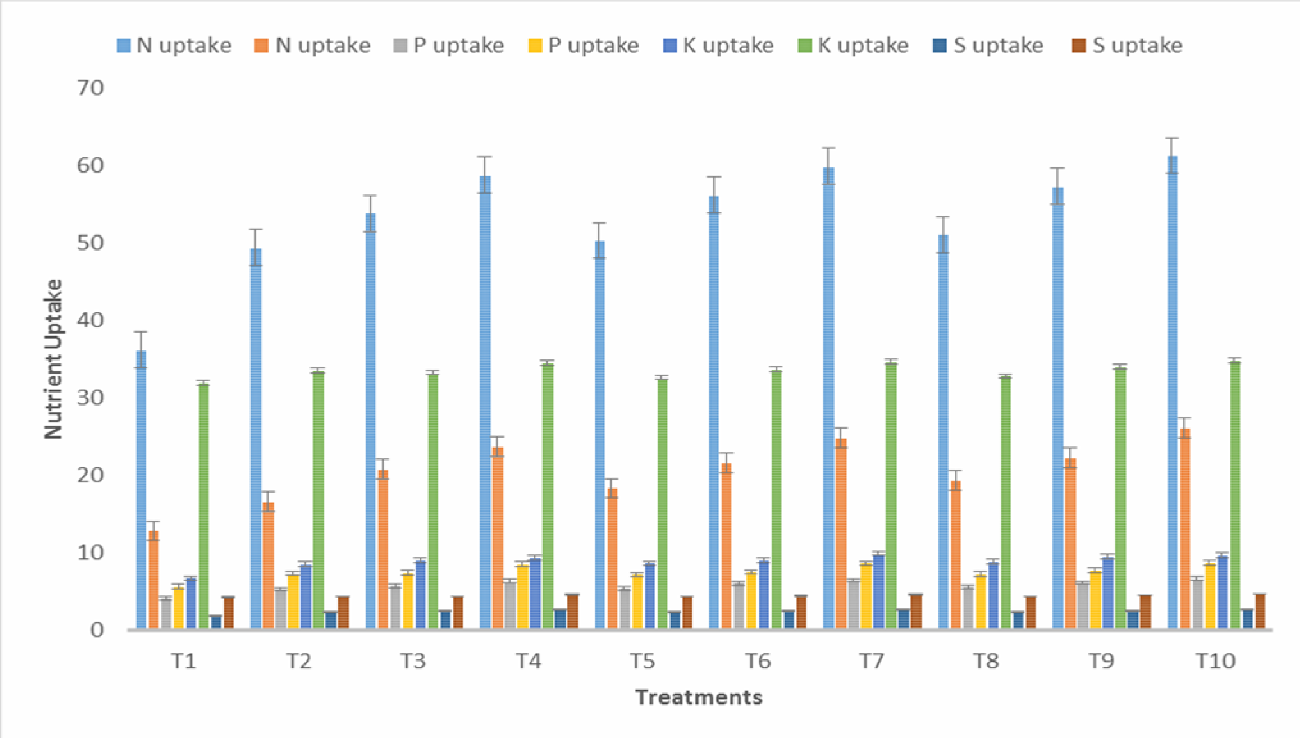

Fig. 2 : Nutrient uptake in chickpea

Quality parameter

Protein content (%)

The protein content of chickpeas was dramatically affected by seed inoculation with Molybdenum in combination with *Rhizobium* and PSB. The application of treatment T10 (*Rhizobium* + PSB + molybdenum at 1 g/kg seed) resulted in a considerably higher protein content of 22.86% (Table 3 and figure 3). The significant rise in protein content in seeds is attributed to the supplemented availability and absorption of nitrogen facilitated by enhanced biological nitrogen fixation, which is positively influenced by bio-fertilizers and molybdenum supplementation, through the efficient functioning of nitrogenase in root nodules and nitrate reductase enzymes within the plant system. This results in more nitrogen availability for the plant, hence enhancing protein synthesis. Chandra and Kothari (2002), Deo *et. al.* 2002), and Elsheikh *et. al.* (2001) also found an increase in seed protein content resulting from the application of molybdenum and bio-fertilizers.

Table 3 : Protein content (%)

Treatments	Protein content (%)
T_1 - Control	17.16
T_2 - 10 g *Rhizobium* + 20g PSB kg^{-1} seed	18.76
T_3 - 15 g *Rhizobium* + 20g PSB kg^{-1} seed	18.33
T_4 - 20 g *Rhizobium* + 20g PSB kg^{-1} seed	21.58
T_5 - 10g *Rhizobium* +20g PSB kg^{-1} seed + AM (0.5g kg^{-1} seed)	19.74
T_6 - 15g *Rhizobium* + 20g PSB kg^{-1} seed + AM (0.5g kg^{-1} seed)	20.14
T_7 - 20g *Rhizobium* + 20g PSB kg^{-1}seed +AM (0.5g kg^{-1} seed)	22.16
T_8 - 10g *Rhizobium* + 20g PSB kg^{-1} seed + AM (1g kg^{-1} seed)	19.86
T_9 - 15g *Rhizobium* + 20g PSB kg^{-1} seed + AM (1g kg^{-1} seed)	20.83
T_{10} - 20g *Rhizobium* + 20g PSB kg^{-1} seed + AM (1g kg^{-1} seed)	22.86
SEm±	0.32
CD at 5%	0.95

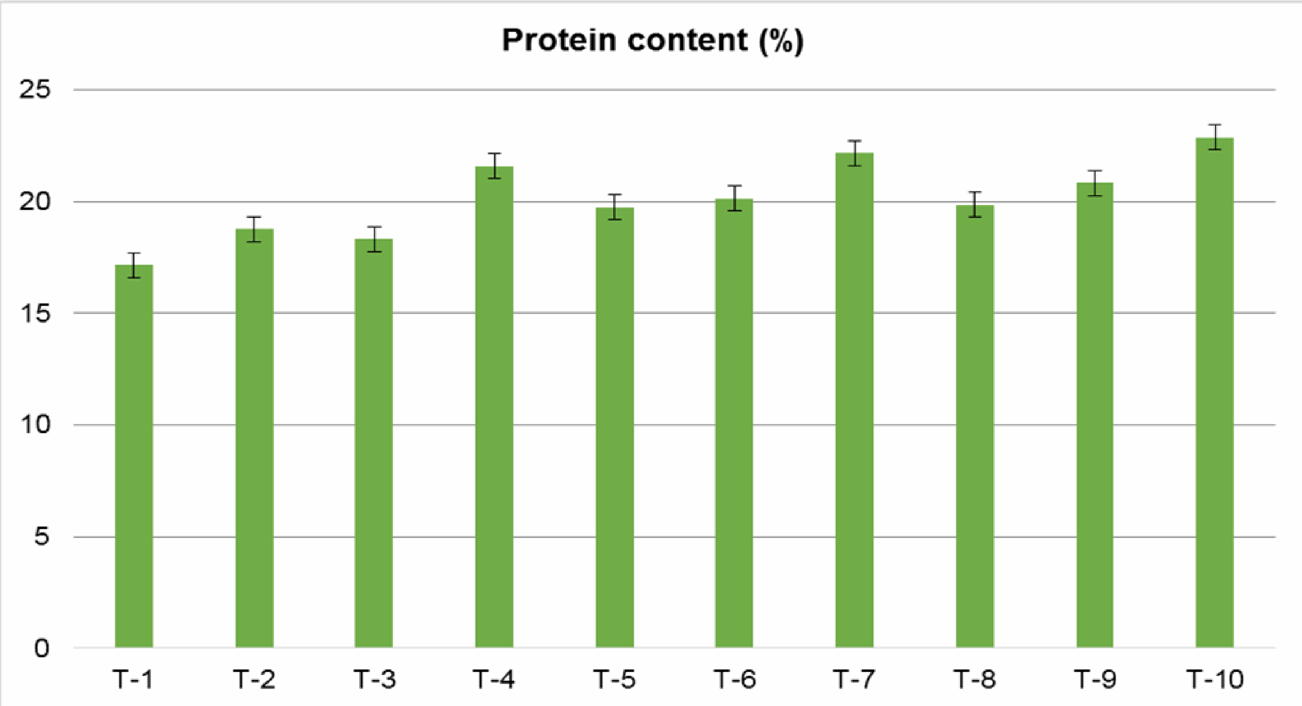

Fig. 3 : Protein content (%)

Conclusion

The study concluded that the combined application of *Rhizobium* and PSB with molybdenum significantly enhances biomass production, nutrient uptake, particularly nitrogen and phosphorus, and improves the quality of chickpea grains by increasing protein content. The biofertilizers, when integrated with molybdenum, not only promoted better nodulation and plant growth but also resulted in higher Seed yield under rainfed conditions in Vertisols. This combination proves to be a promising strategy for sustainable chickpea production, offering a viable solution for enhancing soil fertility and crop quality, especially in low-input agricultural systems. The findings highlight the potential of biofertilizer and molybdenum application as an effective means to improve both productivity and quality of chickpea, contributing to more sustainable agricultural practices.

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