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EFFECT OF RHIZOBIUM AND MOLYBDENUM APPLICATION ON GROWTH AND YIELD RELATED ATTRIBUTES OF LENTIL CULTIVARS

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Abstract

A field experiment was conducted during the Rabi season 2022–23 at the Agronomy Research Farm, Ghazi University, Dera Ghazi Khan, Pakistan, to evaluate the effect of rhizobium inoculation and molybdenum application on the growth and yield attributes of lentil. The experiment was laid out in a factorial Randomized Complete Block Design (RCBD) with three replications using two lentil varieties (Punjab Masoor-2019 and Punjab Masoor-2020). Four treatments were evaluated: T_1 = control, T_2 = sodium molybdate @ 1 g kg^{-1} seed, T_3 = rhizobium inoculation, and T_4 = rhizobium + sodium molybdate @ 1 g kg^{-1} seed. The analysis of variance (ANOVA) indicated significant differences ($P \leq 0.05$) among treatments for most growth and yield parameters. Significant interaction effects between lentil cultivars and treatments were also observed for several yield-related traits ($P \leq 0.05$). Results showed that the combined application (T_4) significantly improved growth and yield parameters compared to individual applications and the control. Maximum plant height, pods per plant, nodulation, and grain yield were recorded under the combined rhizobium + molybdenum treatment. The results indicate that the combined application significantly enhances lentil productivity and biological nitrogen fixation under semi-arid conditions.

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is an important cool-season grain legume cultivated widely in South Asia, West Asia, North Africa and other semi-arid and temperate regions. It is valued as an important source of affordable plant-based protein, carbohydrates, minerals, dietary fibre and other bioactive compounds. Lentil seed generally contains approximately 20–30% protein, with a favourable nutritional profile that makes it an important component of diets, particularly in developing countries where pulses contribute substantially to dietary protein (Wang & Daun, 2006). In addition to its nutritional importance, lentil has considerable agronomic value because of its relatively low input requirement and ability to grow under moisture-limited conditions. In Pakistan, lentil is an important pulse crop, particularly in Punjab, where several improved varieties are recommended for cultivation (Kaale et al., 2023). The Punjab Agriculture Department lists Punjab Masoor-2019 among the recommended lentil varieties for cultivation across Punjab.

As a leguminous crop, lentil can contribute to the sustainability and productivity of cropping systems through biological nitrogen fixation (BNF). Atmospheric nitrogen (N_2), which cannot be directly utilized by plants, is converted into biologically useful nitrogenous compounds through the symbiotic association between legumes and compatible soil bacteria. In lentil, effective association with *Rhizobium* enables the formation of

root nodules, where biological nitrogen fixation takes place. Consequently, adequate nodulation and efficient nitrogen fixation are important determinants of lentil growth (Rehan et al., 2024), biomass production and grain yield (Rehan et al. 2024). Previous field studies have demonstrated that inoculation with effective *Rhizobium* strains can substantially improve nodulation and grain yield of lentil compared with uninoculated plants. For example, inoculation with effective *Rhizobium* strains increased lentil grain yield under field conditions, with the response depending on the effectiveness of the bacterial strain and soil environment (Miller et al., 2024).

The effectiveness of biological nitrogen fixation is influenced by several environmental and nutritional factors. Nutrient availability, soil reaction, soil moisture, temperature and the compatibility between host genotype and bacterial strain can all influence nodulation and nitrogen fixation. Therefore, simply introducing *Rhizobium* into the soil or onto seed does not necessarily guarantee maximum biological nitrogen fixation. Appropriate nutritional conditions are required for the establishment and functioning of the legume–rhizobium symbiosis. Studies conducted on lentil have shown that *Rhizobium* inoculation can increase nodulation, biomass production and grain yield, particularly where the native rhizobial population is absent or ineffective (Czopek et al., 2025).

Among micronutrients, molybdenum (Mo) has particular importance in legumes because of its direct involvement in nitrogen metabolism. Molybdenum functions as a component of the molybdenum-containing cofactors associated with enzymes involved in nitrogen metabolism, particularly nitrogenase and nitrate reductase. Nitrogenase is essential for biological nitrogen fixation, whereas nitrate reductase is involved in the assimilation of inorganic nitrogen into plant metabolic pathways. Thus, adequate Mo nutrition can support both nitrogen fixation and subsequent nitrogen utilization by plants. A recent review of Mo nutrition in grain legumes emphasized that Mo acts as a cofactor for nitrogenase and nitrate reductase and plays an important role in nodulation, biological nitrogen fixation, nitrogen metabolism and protein synthesis (Wang et al., 2024).

The importance of molybdenum in lentil production has been demonstrated experimentally. Togay et al. (2015) reported that Mo application affected yield and yield-related characteristics of lentil, demonstrating the potential importance of Mo nutrition for improving lentil productivity. Similarly, research on lentil has shown that Mo application can influence nodulation and other growth and characteristics, although the magnitude of the response may vary with application method, Mo concentration, soil fertility and environmental conditions. These findings indicate that Mo management should be considered in relation to the overall nutritional and biological environment of the crop rather than as an isolated micronutrient intervention.

The interaction between Mo nutrition and *Rhizobium* inoculation is particularly important because nitrogen fixation depends on the activity of the nitrogenase enzyme within root nodules. Consequently, providing an effective rhizobial population together with adequate Mo may create a favourable environment for efficient biological nitrogen fixation. Experimental evidence supports this interaction (Kalita et al., 2023). A field study conducted over multiple seasons in lentil reported that seed inoculation with *Rhizobium* together with sodium molybdate increased bacterial population, nitrogen uptake, yield attributes and grain yield compared with separate or untreated applications. The combination of *Rhizobium* inoculation and sodium molybdate at appropriate rates produced particularly strong responses in plant height, pods per plant, seeds per pod, seed weight and grain yield. Similarly, integrated use of *Rhizobium* with other nutrient-management practices has been shown to improve lentil performance. Singh et al. reported that *Rhizobium* and plant growth-promoting rhizobacteria improved nodulation, nodule dry weight, leghemoglobin content, chlorophyll concentration, yield attributes and grain yield when used with appropriate phosphorus management. Earlier research under South Asian conditions also demonstrated that *Rhizobium* inoculation increased nodulation, grain yield and biomass protein production of lentil. These findings suggest that integration of biological inoculation with appropriate micronutrient management may provide an effective approach for improving the productivity and nitrogen economy of lentils (Singh et al., 2018).

Despite the demonstrated importance of *Rhizobium* and Mo in legume nutrition, the response of lentil to these inputs can vary considerably according to cultivar, soil properties, indigenous rhizobial populations, environmental conditions and

management practices. This is particularly relevant in semi-arid regions, where high temperatures, limited rainfall and variable soil fertility can restrict crop growth and biological nitrogen fixation (Basit et al., 2024). Dera Ghazi Khan represents an important semi-arid agro-ecological environment where optimization of nutrient and biological inputs is necessary for improving pulse crop productivity. However, comparatively limited information is available regarding the interactive effects of *Rhizobium* inoculation and Mo application on different lentil cultivars under the agro-climatic conditions of Dera Ghazi Khan.

Therefore, evaluating varietal responses to *Rhizobium* and Mo is important for identifying management combinations capable of improving nodulation, vegetative growth, yield components and final grain yield. Such information may also contribute to more efficient nitrogen management by enhancing biological nitrogen fixation and reducing dependence on external nitrogen inputs. The present study was therefore conducted to evaluate the individual and combined effects of *Rhizobium* inoculation and molybdenum application on growth, nodulation, yield components and grain yield of two lentil varieties under the agro-climatic conditions of Dera Ghazi Khan, Pakistan.

Methods

The field experiment was conducted during the Rabi season of 2022–23 at the Agronomy Research Farm, Ghazi University, Dera Ghazi Khan, Pakistan. Pre-sowing composite soil samples collected at a depth of 0–30 cm revealed a sandy loam texture. The soil environment was characterized as slightly alkaline with a pH of 8.1, electrical conductivity (EC) of 1.42 dS m^{-1}, organic matter content of 0.61%, available phosphorus of 6.2 mg kg^{-1}, and extractable potassium of 118 mg kg^{-1}.

The study utilized a factorial Randomized Complete Block Design (RCBD) with three replications. Two distinct lentil varieties were tested as Factor A:

- V_1: Punjab Masoor-2019
- V_2: Punjab Masoor-2020

Factor B comprised four distinct nutrient treatments:

- T_1: Control (No inoculation, no micronutrient application)
- T_2: Molybdenum application alone (1 g kg^{-1} seed)
- T_3: Rhizobium inoculation alone
- T_4: Combined Rhizobium + Molybdenum application

The individual plot size was set to 3.0 m × 1.5 m. Sowing was completed by manual line sowing keeping a row-to-row spacing of 30 cm and a plant-to-plant spacing of 10 cm. The molybdenum source chosen for this study was analytical-grade sodium molybdate, applied uniformly as a dry seed dressing rate of 1 g kg^{-1} of seed. The specific rhizobium inoculum strain utilized was *Rhizobium leguminosarum* biovar, procured from the Soil Bacteriology Section, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. For the inoculation treatments (T_3 and T_4), seeds were uniformly coated using a 10% sugar solution as a sticky adhesive agent to ensure proper bacterial adhesion to the seed coat. Coated seeds were dried in the shade briefly before immediately sowing in the field.

A recommended basal fertilizer dose consisting of 22 kg N ha^{-1} and 57 kg P_2O_5 ha^{-1} was applied uniformly to all plots during land preparation using Diammonium Phosphate (DAP) and Urea to satisfy basic early-stage starter requirements. All other agronomic practices, including three uniform irrigations applied at the vegetative, flowering, and pod formation developmental stages, alongside manual weeding, were maintained identically across all treatment blocks. Data collected for growth parameters, nodulation, and yield attributes were organized and subjected to a two-way analysis of variance (ANOVA) using Statistix 8.1 software. Main effects of treatments, varietal differences, and their interactive combinations were tested. Treatment means were statistically differentiated and compared using the Least Significant Difference (LSD) test at a 5% ($P \leq 0.05$) level of probability.

Results

Growth and yield attributes

The standalone and integrated applications of rhizobium and molybdenum significantly ($P \leq 0.05$) influenced the vegetative development, root nodulation, and yield-forming parameters of lentil (Table 1). Analysis of variance indicated that varietal differences between Punjab Masoor-2019 and Punjab Masoor-2020 were statistically significant for plant height and final grain yield, with Punjab Masoor-2020 expressing a slightly superior performance profile overall. Furthermore, the interactive effects between cultivars and treatment

combinations were also found to be significant ($P \leq 0.05$), showing that the concurrent application of both biological and micronutrient inputs yielded maximum performance across both crop varieties.

The combined treatment (T_4) generated the highest performance values across all recorded growth parameters, producing a plant height of 39.23 cm, 4.40 branches per plant, and 13.63 nodules per plant. These gains represent an explicit enhancement in vegetative capacity and general crop vigor compared to the un-inoculated control (T_1), which recorded a plant height of only 28.93 cm and 2.67 branches per plant.

The significant elevation in nodule count per plant from 5.97 in the control up to 13.63 in T_4 strongly confirms that external rhizobium introduction successfully ensures a highly competitive population of effective bacteria in the rhizosphere. This finding is completely consistent with earlier reports noting that purposeful seed inoculation triggers efficient radical infection and subsequent nodule formation due to improved nitrogen fixation dynamics (Ahmed et al. 2008; Das et al. 2017).

The distinct positive response observed with the addition of molybdenum is deeply rooted in the structural biochemistry of the legume-rhizobium symbiosis. Molybdenum operates as a fundamental catalytic core of nitrogenase, the multi-subunit enzyme complex responsible for breaking the triple chemical bond of atmospheric N_2 (Hernandez et al. 2009; González-Guerrero et al. 2014). When molybdenum is applied as a seed treatment, it rapidly assimilates into developing root tissue, satisfying the high Mo demand of the bacterial bacteroids inside root nodules. This directly enhances nitrogenase enzyme synthesis, optimizing biological nitrogen fixation and maximizing the structural assimilation of nitrogen compounds early in the plant life cycle (Hernandez et al. 2009; González-Guerrero et al. 2014). Furthermore, molybdenum plays an identical cofactor role in nitrate reductase, an essential plant enzyme required to reduce taking up soil nitrates into usable proteins (Hernandez et al. 2009; González-Guerrero et al. 2014). This dual enzymatic stimulation is responsible for the substantial boosts observed in biomass collection, plant height, and general vegetative branching.

Yield performance

Grain yield and yield components were significantly improved by combined application (Table 1). The highest grain yield (522.22 kg ha^{-1}) and 1000-grain weight (37.45 g) were observed under T_4, while the lowest values were recorded in the control treatment. Conversely, the control treatment remained significantly lower, producing the lowest overall grain yield at 240.74 kg ha^{-1}. The synergistic interaction between rhizobium and molybdenum effectively guarantees a continuous, synchronized supply of fixed nitrogen during critical post-anthesis grain filling stages (González-Guerrero et al. 2014). This steady nitrogen pool enhances the source-to-sink mobilization of photoassimilates, increasing seed weight and final grain output.

The synergistic effect of rhizobium and molybdenum enhances nitrogen fixation efficiency and biomass production (González-Guerrero et al. 2014). Similar results have been reported by Singh et al. (2014) and Togay et al. (2015).

Table 1. Effect of rhizobium and molybdenum application on growth and yield attributes of lentil (mean ± SE).

Treatment	Plant height (cm)	Pods $plant^{-1}$	Seeds pod^{-1}	Branches $plant^{-1}$	Nodules $plant^{-1}$	1000-grain weight (g)	Grain yield (kg ha^{-1})
T_1 (Control)	28.93 ± 0.68	15.00 ± 1.28	1.33 ± 0.05	2.67 ± 0.07	5.97 ± 0.24	33.04 ± 0.17	240.74 ± 10.49
T_2 (Mo)	29.63 ± 0.31	16.33 ± 1.25	1.40 ± 0.00	3.43 ± 0.23	7.73 ± 0.11	33.87 ± 0.34	325.93 ± 23.45
T_3 (Rhizobium)	34.23 ± 2.27	19.50 ± 0.62	1.40 ± 0.05	4.10 ± 0.44	10.02 ± 0.36	35.58 ± 0.47	388.89 ± 13.87
T_4 (Rhizobium + Mo)	**39.23 ± 1.58**	**29.17 ± 1.33**	**1.58 ± 0.06**	**4.40 ± 0.25**	**13.63 ± 0.47**	**37.45 ± 0.77**	**522.22 ± 33.17**

Note: Means followed by different letters differ significantly at $P \leq 0.05$ according to LSD test. Values are treatment means averaged across both varieties; SE = standard error

Discussion

The present study showed that *Rhizobium* inoculation and molybdenum (Mo) application significantly improved growth, nodulation and yield attributes of lentil. The combined application of *Rhizobium* + Mo (T_4) produced the highest plant height (39.23 cm), branches plant^{-1} (4.40) and nodules plant^{-1} (13.63), compared with 28.93 cm, 2.67 branches and 5.97 nodules plant^{-1} in the control. The improved growth under inoculation may be attributed to enhanced biological nitrogen fixation through effective root nodulation, which increases the availability of nitrogen for vegetative development. Similar positive effects of *Rhizobium* inoculation on nodulation and productivity of lentil have been reported by Ahmed et al. (2008).

Molybdenum further enhanced the response to *Rhizobium*. Nodules increased from 10.02 plant^{-1} with *Rhizobium* alone to 13.63 plant^{-1} with *Rhizobium* + Mo. Molybdenum is an important component of the molybdenum cofactor required for nitrogenase and nitrate reductase, making it important for biological nitrogen fixation and nitrogen metabolism in legumes (Hernandez et al., 2009; González-Guerrero et al., 2014). The greater nodulation under combined treatment therefore suggests that adequate Mo availability supported the effectiveness of the lentil–*Rhizobium* symbiosis.

Yield components followed the same trend. The combined treatment produced the highest pods plant^{-1} (29.17), seeds pod^{-1} (1.58) and 1000-grain weight (37.45 g), compared with 15.00, 1.33 and 33.04 g, respectively, under the control. Consequently, grain yield increased from 240.74 kg ha^{-1} in the control to 522.22 kg ha^{-1} under *Rhizobium* + Mo, an increase of approximately 117%. The greater yield was therefore associated with simultaneous improvement in nodulation and yield components. Similar beneficial effects of Mo and *Rhizobium* on lentil growth, nodulation, N uptake and yield have been reported previously (Togay et al., 2015; Singh et al., 2018; Kalita et al., 2023).

The significant varietal differences for plant height and grain yield indicate genetic variation in the response of Punjab Masoor-2019 and Punjab Masoor-2020, while the significant variety × treatment interaction suggests that the effectiveness of *Rhizobium* and Mo depended partly on cultivar. Overall, the results indicate that combined *Rhizobium* inoculation and Mo application was more effective than either input alone for improving lentil growth, nodulation and grain yield under the conditions of Dera Ghazi Khan.

Conclusion

The combined application of rhizobium inoculation and molybdenum (via sodium molybdate seed dressing @ 1 g kg^{-1} seed) significantly improved growth, nodulation, and yield of lentil under the agro-climatic conditions of D.G. Khan. This integrated nutrient management approach enhances biological nitrogen fixation and nutrient efficiency, offering a sustainable strategy for improving lentil productivity under semi-arid conditions.

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