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Soybean Productivity and Nutritional Quality as Influenced by Graded Phosphorus and Molybdenum Fertilization under *Typic Hapludalfs*

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Abstract: The present investigation was aimed to evaluate graded P (0, 30, 60 and 90 kg ha^{-1}) and Mo (0,1 and 2 kg ha^{-1}) levels through 12 treatment combinations on the yield, nutrient uptake and quality of soybean. The highest grain yield, NPK and Mo uptake was with highest application rates of both the tested nutrients. The protein content and protein yield also recorded remarkable improvements with the same treatments. The conjoint application of 90 and 2 kg ha^{-1} P and Mo exhibited a significant impact on number of pods per plant^{-1} and Mo uptake by grain. The study concluded that higher soybean yield with enhanced quality under prevailing acidic soil conditions, marked by P fixation and Mo deficiency can be achieved by applying these nutrients at their peak tested rates.

Keywords: Phosphorus, Molybdenum, Soybean yield, Nutrient uptake, Protein content

Heavy dependence solely on macronutrient fertilizers initially boosted the short-term crop yields but caused long-term soil degradation. Avoiding micronutrients' application disrupted the nutrient balance within the interconnected soil-plant-animal-human continuum. Amidst macronutrients, phosphorus (P), classified as the second most limiting after nitrogen in Indian soils, but plays crucial roles in energy production, signaling, homeostasis and nucleic acid synthesis (DNA/RNA) in humans (Shehzad et al., 2022). Despite regular/continuous application of phosphatic fertilizers to increase crop yields, P availability is often low, due to high affinity for the soil solid phase (Zhu et al., 2018). Concurrently, micronutrient deficiencies are widespread in India, particularly in lower-middle-income populations with calorie-dense, nutrient-poor diets. Among these, molybdenum (Mo), an essential element for humans, act as a cofactor for several enzymes essential for metabolism, cell signaling, differentiation and antioxidant defense has been reported deficient in about 13% of Indian soils impacting the soil- plant-human health (Behera et al., 2014).

In India, food and nutritional insecurity is on the rise particularly in the increasing demographic scenario and changing agro-climatic conditions. Under such situations, soybean (*Glycine max* L.), though a non-staple crop offers the inexpensive plant-based source of high-quality protein, rich in essential amino acids, micronutrients and vitamins, vital in combating malnutrition and ensuring food and nutritional security (Rashid et al., 2023). Above all, soybean's added advantage having remarkable ability to biologically fix nitrogen partially offsets the need for synthetic nitrogen fertilizers, emphasizing its importance in arresting the negative soil and environmental implications.

However, being nutrient intensive crop, often faces multi-nutrient deficiencies across major growing regions worldwide. Soybean specifically relies on adequate Mo fertilization to achieve maximum yield potential. However, non-replenishment of adequate Mo is causing its deficiency and significant yield and quality losses, severely impairing N-fixation, a cornerstone of soybean productivity (Zhang et al., 2023).

Deficiencies of both these nutrients exacerbates under humid subtropical climate with monsoonal influences matching the experimental site (Palampur region) where soils have been classified as *Typic Hapludalfs,* moderate to low in fertility and high susceptibility to nutrient leaching, exhibiting poor P availability locked away by sorption, precipitation (typically by reaction with Al^{3+} and Fe^{3+}) or microbial immobilization resulting in low P use efficiency and decline in soybean yield up to 29-45% (Vishwanath et al., 2022). Owing to acidic conditions, molybdenum is bound to be insufficient. It is involved in diversified functions and acts as a key to unlock nitrogen fixation in legumes like soybean. At low pH, the $HMoO_4$ is absorbed by silicate clays and oxides of Fe and Al through ligand exchange with hydroxide ions on the surface of the colloidal particles which hinders its availability. Oliveira et al. (2022) suggested that Mo concentrations below 0.1 ppm in mature leaf tissues in various crops indicate its deficiency, restricted flower development and nodule formation (Rana et al., 2020). However, limited research has been done with conjoint application of P and Mo on soybean under *Typic Hapludalfs*, hence this investigation was aimed with the hypothesis that graded P and Mo levels will enhance productivity, quality, and nutrient uptake by soybean.

MATERIAL AND METHODS

Study site: The present study was undertaken on soybean during *kharif* 2021, at CSK HP Agriculture University, Palampur (32° 6' N latitude and 76° 3' E longitude, 1290 m amsl). The site lies within the mid hill wet temperate zone (Zone II) of HP and is characterized as *Typic Hapludalfs*, with silty clay loam texture and pH of 5.32. During the experimental period, the crop received a total rainfall of 1882 mm, with mean maximum and minimum temperatures of 31.1 and 10.5°C, respectively and weekly relative humidity varying from 60 to 94.03%. The soil properties at the initiation of the experiment have been depicted in Table 1.

Experimental details: The experiment was arranged in randomized block design using 4 levels of P *viz.,* P_0: 0, P_1: 30 P_2: 60 and P_3: 90 kg P_2O_5 ha^{-1} and 3 levels of Mo *viz.,* Mo_0:0, Mo_1: 1 and Mo_2: 2 kg Mo ha^{-1}, thus, having 12 treatment combinations (T_1: P_0Mo_0, T_2: P_0Mo_1, T_3: P_0Mo_2, T_4: P_1Mo_0, T_5: P_1Mo_1, T_6: P_1Mo_2, T_7: P_2Mo_0, T_8: P_2Mo_1, T_9: P_2Mo_2, T_{10}: P_3Mo_0, T_{11}: P_3Mo_1 and T_{12}: P_3Mo_2) replicated thrice. Basal applications of 20 kg N and 40 kg K_2O ha^{-1} through urea and MOP while, P and Mo as per treatment structure were done through SSP and sodium molybdate, respectively. The standard agronomic measures were followed during the entire growth period of the crop.

Sampling and analysis: After harvest, the plant samples (grain and straw) were collected, dried in an oven at 60 °C, grounded in a Wiley mill and stored in moisture free paper bags for further analysis. The protein content and protein yield of the digested samples was worked out.

Protein content (%)= N content (%) x 6.25 (Walinga et al., 1989)

Protein yield (kg ha^{-1}) = Grain yield (kg ha^{-1}) x Protein content (%) / 100

Whereas, the nutrient uptakes were calculated using the following formulae:

Nutrient uptake by grain (kg ha^{-1}) = Nutrient content in grain (%) x Grain yield (q ha^{-1})

Nutrient uptake by straw (kg ha^{-1}) = Nutrient content in straw (%) x Straw yield (q ha^{-1})

Statistical analysis: The data was analyzed using the technique of analysis of variance for randomized block design (Gomez and Gomez 1984) using Microsoft Excel.

RESULTS AND DISCUSSION

Yield Attributes

Number of pods $plant^{-1}$: The individual application of P and Mo along with their interaction effect, significantly increased the pod count. The exclusive application of P and Mo at respective highest rates exhibited a significant edge over the lower levels (Table 2). With the graded P application, the pod count followed the trend 90 > 60 > 30 > 0 kg P ha^{-1}. However, the control treatment showed diminution of about 27 and 20 % over 90 and 60 kg P ha^{-1}, respectively. In contrast, Manoj et al. (2023) recorded maximum pod count in soybean with 40 kg P ha^{-1}. The adequate P fertilization in the regions prone to its high fixation, perfectly matching our situations (acid soils), might have improved its availability during the early crop growth stages (Johan et al., 2021). In a comparable manner, the progressive reductions in Mo doses at each level significantly reduced the pod number. But, with raised dose from 0 to 2 kg Mo ha^{-1}, increase from 73.5 to 80.4, respectively was observed, emphasizing the significance of Mo application addressing its deficiency at the experimental site. The application of 1 and 2 kg Mo ha^{-1} demonstrated noteworthy increases of about 5 and 10 %, respectively over the control. The probable reason could be the efficient use of Mo applied at the time of sowing, regulated effective nodulation, nitrogen fixation and contributed to better performance. Padhi and Pattanayak (2018) also observed maximum enhancement in yield attributes of mungbean with the use of Mo. Additionally, omitting phosphorus resulted in a more significant reduction in the pod number (63.0) compared to the exclusion of molybdenum (73.4) highlighting more reliance on P.

Interaction of P and Mo on number of pods $plant^{-1}$: The conjoint use of P and Mo exerted a synergistic effect on the pod count and the highest was recorded with 90 kg P ha^{-1} and 2 kg Mo ha^{-1} (88.9) trailed by 90 kg P ha^{-1} and 1 kg Mo ha^{-1}

Table 1. Initial characteristics of the soil

Soil property	Value
Mechanical separates (%)	
Sand	19.30
Silt	44.60
Clay	34.40
Textural class	Silty clay loam
Chemical analysis	
Soil pH (1:2.5)	5.32
Organic carbon (g kg^{-1})	7.31
Available nutrients (kg ha^{-1})	
N	307
P	16.3
K	119
Mo (ppm)	0.160

(85.4) and the least under control (61.8) (Table 3). The response of Mo to graded P application highlighted that in the absence of P, a consistent increase was up to 2 kg ha^{-1}. Conversely, at 30 and 60 kg P ha^{-1}, significant variations were observed only at 2 kg Mo ha^{-1} but, the use of 90 kg P ha^{-1} recorded a consistent increase up to 2 kg Mo ha^{-1}. Likewise, the performance of P under varied Mo doses revealed that with the omission of Mo, a marked increase was documented only up to 60 kg P ha^{-1}. But, at 1.0 and 2.0 kg Mo ha^{-1}, a steady increase up to 90 kg P ha^{-1} was observed. Ahmad et al. (2022) also reported significantly higher pods $plant^{-1}$ in mung bean with the conjoint use of 60 kg P ha^{-1} and 1.5 kg Mo ha^{-1}.

Number of seeds pod^{-1}: Significant variations in number of seeds pod^{-1} were observed in response to the graded applications of P and Mo (Table 2). The use of 30, 60 and 90 kg P ha^{-1} demonstrated improvements of about 8, 10 and 12 %, respectively over control. This enhancement might be attributed to the vital role of P in improving photosynthesis, root expansion, carbohydrate transfer, increasing sink capacity through improved flowering and metabolite transfer to pods, thereby aiding the seed establishment (Nikfarjam and Aminpanah 2015). Whereas, scarcity can lead to reduced seed number, as evidenced from the significant drop in the control. Shenoy and Kalagudi (2005) also highlighted that the deficiency of P can reduce the soybean yield by about 10-15%. Khan et al. (2017) observed higher seed count per pod compared to lower P rates. The 2 kg Mo ha^{-1} resulted in highest count (2.66) owing to the better supply of this deficient nutrient. In contrast, the lowest number was observed under Mo-devoid treatment (2.55), registering a diminution of about 3 and 4% over 1 and 2 kg Mo ha^{-1}, respectively. The significant reduction in the seed count pod^{-1} (2.42) was with the exclusion of P compared to the omission of Mo (2.55). These results corroborate the findings of Oliveria et al. (2022). However, the conjoint use of both the nutrients showed statistical equivalence.

Grain yield: The significant increase in the grain yield was attained with the graded application of both the nutrients (P and Mo) (Table 2), though their interaction exhibited no significant impact. Among the P levels, the yield response followed the trend 90 > 60 > 30 kg P ha^{-1} and registered an increase of about 61, 45, and 26 % over the control, respectively, underscoring the positive impact of P fertilization in acid soils exhibiting high fixation of phosphate radicals by acidic cations (Chen et al., 2022) or their complexation with soil organic matter and microorganisms (Huang et al., 2017). Adequate P improves the rhizosphere nutritional environment, accelerates root growth, flowering, seed formation, facilitates translocation of stored assimilates to seeds, ultimately aids in boosting the crop productivity (Khan et al., 2020). Similarly, the reductions in Mo doses at each level documented a declining trend in the yield and the control registered a drop of about 10 and 19 % over 1 and 2 kg Mo ha^{-1}, respectively. The proportionate increase with the graded doses might be owing to its predominant role in the biological nitrogen fixation, enhancing nitrogen absorption

Table 3. Interaction effect of graded P and Mo application on number of pods $plant^{-1}$

Treatment	Phosphorus levels			
Molybdenum levels	P_0	P_1	P_2	P_3
Mo_0	61.8	72.7	78.2	81.4
Mo_1	65.7	74.9	80.9	85.4
Mo_2	73.4	77.9	81.5	88.9
CD (p≤0.05)	3.4			

Table 2. Impact of graded P and Mo application on yield attributes, yield and quality parameters of soybean

Treatment	No. of pods $plant^{-1}$	No. of seeds pod^{-1}	Grain yield (tonnes ha^{-1})	Protein content (%)	Protein yield (q ha^{-1})
Phosphorus levels					
P_0	67.0	2.42	1.54	34.4	4.81
P_1	75.2	2.61	1.94	35.7	6.04
P_2	80.2	2.65	2.23	37.8	8.52
P_3	85.2	2.72	2.48	38.4	9.96
CD (p≤0.05)	2.0	0.03	0.06	1.6	0.29
Molybdenum levels					
Mo_0	73.5	2.55	1.87	35.4	6.83
Mo_1	76.7	2.58	2.05	36.8	7.33
Mo_2	80.4	2.66	2.21	37.5	7.84
CD (p≤0.05)	1.7	0.03	0.05	1.3	0.25

thereby the yield (Guo et al., 2023). The increased Mo application under the deficient conditions might also have elevated the concentration in the soil solution for the uptake. The omission of P resulted in a greater yield reduction over Mo. Oliveira et al. (2017) also highlighted a significant impact of P and Mo fertilization on the soybean yield.

Quality Attributes

Protein content and protein yield: The individual factors (P and Mo) exerted a significant influence on the protein content and protein yield (Table 2), whereas their conjoint impact failed to register any significant improvement. The maximum protein content was with 90 kg P ha^{-1} (38.4 %) which showed statistical parity with 60 kg P ha^{-1} (37.8%) and the lowest under control (34.4%). The applications at 30, 60 and 90 kg P ha^{-1} increased the content by 4, 10 and 12%, respectively over control owing to the complementary relationship between nitrogen and phosphorus; which aids in the formation of stable phospho-protein compounds as nitrogen facilitates amino acid synthesis while phosphorus supports energy intensive processes such as nitrogen fixation, uptake and assimilation (Zhao et al., 2023). The addition of sulphur through SSP might have contributed significantly in protein synthesis as S helps in the formation of disulfide bonds which have paramount role in formulating and regulating the stability and configuration of the protein structures (Patel et al., 2019). The reductions in Mo doses registered a decline in the protein content and the lowest was documented under control (35.4 %) followed by 1 kg Mo ha^{-1} (36.8%) which behaved statistically alike with 2 kg Mo ha^{-1} (37.5%). The treatment devoid of Mo, reduced the content by about 6% over 2 kg Mo ha^{-1}. As Mo is a vital component of dual enzymes, nitrate reductase and nitrogenase; helps in producing more nitrogen through nitrate reduction and fixation respectively, leading to enhanced protein content in seeds (Ahmad et al., 2022). In case of protein yield, skipping both P and Mo led to substantial reductions in protein yields over their peak levels, but was more pronounced with P omission (about 107%) compared to Mo (about 15 %). The use of 30, 60 and 90 kg P ha^{-1} yielded about 6.04, 8.52 and 9.96 q ha^{-1} protein, respectively. The application of 1 and 2 kg Mo ha^{-1} produced 7.33 and 7.84 q ha^{-1} protein yields, respectively. The quantum jump with the highest levels of both P and Mo could be ascribed to the higher protein content and grain yields. The findings are in total agreement with Singh et al. (2017) in lentil advocating the same dose, however, Khaswa et al. (2014) recorded higher values with 40 kg P ha^{-1} in soybean.

Nutrient Uptake

Nitrogen uptake: Increased P and Mo rates exhibited significant improvements in nitrogen uptake by both grain and straw over rest of the treatments (Table 4). However, their conjoint application did not exhibit any significant interaction. Application of 90 kg P ha^{-1} demonstrated peak uptakes by both grain (159.4 kg ha^{-1}) and straw (26.9 kg ha^{-1}) followed by 60 (136.4 and 23.6 kg ha^{-1}) and 30 (96.7 and 19.7 kg ha^{-1}) kg P ha^{-1} registering improvements of about 107 and 65 % by grain and straw, respectively over respective controls as the uptake is a function of concentration within the plant and the overall crop yield (Gourav et al., 2019). P, being energy currency plays essential role in improving photosynthesis and metabolic processes, collectively boosting the grain nitrogen content. The improved grain yield with elevated P application could be ascribed to the better nutrient absorption, greater dry matter accumulation and

Table 4. Influence of varied P and Mo applications on the nutrient uptake by grain and straw

Treatment	Nitrogen uptake (kg ha^{-1})		Phosphorus uptake (kg ha^{-1})		Potassium uptake (kg ha^{-1})		Molybdenum uptake (g ha^{-1})	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
Phosphorus levels								
P_0	77.0	16.3	5.77	0.92	11.0	32.5	2.93	2.21
P_1	96.7	19.7	7.57	1.76	14.1	40.8	4.16	3.15
P_2	136.4	23.6	9.29	2.41	16.9	46.7	4.68	3.77
P_3	159.4	26.9	11.09	3.41	19.2	52.4	5.70	4.40
CD ($p \le 0.05$)	4.7	1.4	1.3	0.6	1.7	5.2	0.5	0.4
Molybdenum levels								
Mo_0	109.3	19.3	7.12	1.56	12.9	40.3	3.66	2.99
Mo_1	117.4	22.1	8.46	2.14	15.3	42.3	4.36	3.39
Mo_2	125.4	23.5	9.72	2.67	17.7	46.7	5.07	3.80
CD ($p \le 0.05$)	4.1	1.2	1.1	0.5	1.5	4.5	0.4	0.4

effective translocation of photosynthates to the grain (Ahmad et al., 2018, Anwar et al., 2018). The graded Mo levels also behaved alike to the graded P doses and use of 2 kg Mo ha^{-1} accelerated the uptake by both grain and straw (125.4 and 23.5 kg ha^{-1}, respectively) trailed by 1 kg Mo ha^{-1} (117.4 and 22.1 kg ha^{-1}, respectively). The uptake at 2 kg Mo ha^{-1} increased uptakes by about 15 and 22% by grain and straw, respectively over their controls owing to its roles in improving the N metabolism and use efficiency (Probst et al., 2021). As Mo is a key component of enzymes nitrogenase and nitrate reductase, plays pivotal role in nitrogen assimilation and transport which further contribute to its enhanced uptake (Oliveria et al., 2022). Omission of P (77 and 16.3 kg ha^{-1}) caused a greater decline in uptake compared to Mo (109.3 and 19.3 kg ha^{-1}) by grain and straw, respectively.

Phosphorus uptake: Significant variations in P uptake by soybean grain and straw were recorded in response to varied rates of P and Mo (Table 4), but their interaction effect showed statistical equivalence. The uptakes by grain and straw followed the trend 90 > 60 > 30 >0 kg P ha^{-1} and 2 > 1 > 0 kg Mo ha^{-1}. The use of 90 kg P ha^{-1} registered improvements of about 92 and 270 % in grain and straw, respectively, over respective controls. The increased utilization of P by the crop might be due to higher soil P concentration owing to higher addition which likely satisfied the crop's initial nutrient needs thus enhancing nutrients uptake. The low P use efficiency (approximately 15-20%) by most crops in acid soils owing to high fixing capacity leads to accumulation of large amounts of legacy P which prevents plant absorption and necessitates substantial phosphate fertilization (Zhu et al., 2018). The graded Mo application exhibited analogous results to varied P levels. Omitting Mo resulted in decline of about 37 and 71 % by grain and straw respectively, over 2 kg Mo ha^{-1}. Mo stimulates the secretion of root exudates *viz.,* succinic acid, malic acid and acid phosphatase in leguminous crops, thus promoting the conversion of soil-insoluble phosphorus into plant -available forms (Qin et al., 2023). Rana et al. (2020) also emphasized the role of Mo application in enhancing P availability within the rhizosphere soil of leguminous crops and ultimately the crop yields.

Potassium uptake: The potassium uptake was significantly influenced by graded P and Mo doses (Table 4). The peak uptakes by grain and straw were with 90 kg P ha^{-1} (19.2 and 52.4 kg ha^{-1}, respectively) and the minimum under control (11 and 32.5 kg ha^{-1}, respectively). The enhancement might be owing to the increased dry matter production and higher nutrient content in the P- treated plots. P plays a crucial role in stimulating root growth, increasing root surface area for nutrient absorption, providing energy in the form of ATP to support active potassium transport, thereby helped in improving it's uptake. Mo application enhanced grain uptake from 12.9 kg ha^{-1} under control to 17.7 kg ha^{-1} at 2 kg Mo ha^{-1}; accounting for an increase of about 37 %. Conversely, all the Mo doses behaved statistically alike with respect to K uptake by straw, and ranged from 40.3 kg ha^{-1} under control to 46.7 kg ha^{-1} at 2 kg Mo ha^{-1}. The numerical increase might be due to the favorable environmental conditions for growth and biomass production of soybean (Lateef et al., 2021). The interaction effect of both the nutrients exhibited non-significant impact on the uptakes.

Molybdenum uptake: The sole applications of P and Mo exhibited a marked impact on the Mo uptake by grain and straw and was maximum at highest levels (Table 4), though the interaction between the two was significant only in grain (Table 5). An exponential increase in uptake was observed with the increasing P doses. The uptakes at 90 kg P ha^{-1} recorded an increase of about 94 and 99 % in grain and straw, respectively over their controls. Huang et al. (2023) also highlighted the synergistic relationship between P and Mo. The graded application of both the nutrients enhanced their absorption, improved photosynthesis and utilization, higher use efficiency and ultimately yield. Similarly, Mo doses exhibited linear increase in uptake and the use of 2 kg ha^{-1} increased the uptakes in grain and straw by about 38 and 27 %, respectively, over their controls. The reduced uptake in control can likely be attributed to the absence of Mo supplementation and its inherent deficiency in the prevailing acidic soil conditions. The application of graded doses probably elevated its concentration in the soil solution, enhancing availability and subsequent utilization.

Interaction of P and Mo on Mo uptake by grain: The response of Mo to graded P application revealed that in absence of P, a consistent increase in uptake was documented upto 2.0 kg Mo ha^{-1} (Table 5). Whereas, at 30 and 60 kg P ha^{-1}, graded Mo application behaved statistically alike. But, at 90 kg P ha^{-1}, again consistent increase upto 2.0 kg Mo ha^{-1} was observed. Likewise, the response of P to varied Mo levels revealed that when 0 and 1 kg Mo ha^{-1} was applied, significant variations in uptake were observed only upto 30 kg P ha^{-1} which behaved statistically alike with 60 kg P

Table 5. Interaction effect of graded P and Mo applications on the Mo uptake (g ha^{-1}) by grain

Treatment	Phosphorus levels			
Molybdenum levels	P_0	P_1	P_2	P_3
Mo_0	1.74	3.72	4.16	5.01
Mo_1	2.65	4.15	4.83	5.83
Mo_2	4.39	4.62	5.03	6.26
CD (p=0.05)		0.8		

ha^{-1}, but thereafter a significant increase upto 90 kg P ha^{-1} was observed. When 2.0 kg Mo ha^{-1} was applied, a significant response was found only when combined with 90 kg P ha^{-1}. Huang et al. (2023) also highlighted that the co-application of Mo and P strengthens the root system of soybean, leading to enhanced nutrient absorption. Rana et al. (2020) has reported that phosphomolybdate complexes, formed by the synergistic interaction between phosphate and molybdate ions create a readily available form of phosphorus for plants, highlighting the role of Mo in enhancing P uptake in crops.

CONCLUSION

In acid soils, P in spite of having sufficient content experiences fixation, whereas, Mo is inherently deficient. Coupling P and Mo at their highest rates (90 and 2 kg ha^{-1}) enhanced soybean yield, quality attributes and uptakes. The increased quality attributes will certainly be a step to address the nutritional security issues. However, further higher doses may be tested in such soils involving different crops and cropping systems.

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