Journal of Elementology ISSN 1644-2296

Çelik, A., Cun, S., Fırat, Z., Beyyavaş, V., Sakin, E. and Yıldırım, H. (2025)
'Evaluation of the effects of molybdenum on the growth and development of cotton (*Gossypium hirsutum* l.) plant grown in calcareous soils, and on soil enzyme activity',
Journal of Elementology, 30(1), 95-114,
available: https://doi.org/10.5601/jelem.2024.29.3.3394

RECEIVED: 29 August 2024
ACCEPTED: 28 January 2025

ORIGINAL PAPER

Evaluation of the effects of molybdenum on the growth and development of cotton (*Gossypium hirsutum* l.) plant grown in calcareous soils, and on soil enzyme activity*

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Abstract

This study evaluated the effects of molybdenum (Mo) applications at different doses (via seed, foliar, and soil routes) on cotton plant growth and development, and on the soil parameters. The plant traits examined included plant height, fresh and dry weight of the plant, root length, fresh and dry root weight, stomatal conductance, SPAD value, leaf area, while the soil parameters consisted of the activities of soil enzymes (urease, dehydrogenase, and catalase), soil pH, and electrical conductivity (EC). The results demonstrated that Mo applications significantly improved plant height, root length, fresh and dry root weight, as well as fresh and dry plant weight. The greatest improvements in these parameters were induced by an application of 50 μg kg^{-1} Mo to the soil, while the lowest increase was recorded following a foliar application of 25 μg kg^{-1} Mo. The soil application of 50 μg kg^{-1} Mo resulted in significant increases, particularly in plant height, root length, and biomass (fresh and dry weight). In terms of stomatal conductance, SPAD value, and leaf area, increases were observed across all application methods. The highest SPAD value was obtained with a foliar application of 50 μg kg^{-1} Mo, while the most significant increases in stomatal conductance and leaf area were induced by the same dose applied to the soil. Regarding soil enzyme activities, soil application of Mo ensured the highest increases in urease and catalase activities, highlighting its role in the nutrient cycling and organic matter decomposition. The greatest increase in dehydrogenase activity was recorded after a foliar application of 50 μg kg^{-1} Mo. Overall, the findings showed that the most pronounced improvements in plant growth, biomass, and soil enzyme activity parameters were achieved with the soil application of 50 μg kg^{-1} Mo. These results emphasize the critical role of soil-based molybdenum supplementation in optimizing plant growth, enhancing yield, and promoting soil health. Molybdenum is highlighted as a vital component in sustainable agricultural practice and soil fertility management.

Keywords: soil enzymes, plant growth, foliar fertilization, pH, molybdenum

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* This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) stands as one of the foremost economic crops globally, pivotal in fiber production. Cultivated extensively across over 50 countries, cotton serves as a primary source of fiber (Kumar et al. 2023), utilized across diverse sectors such as the oil and feed industries for its seeds and the paper industry for its linter (Munir et al. 2020). Similar to other plants, cotton requires essential nutrients for its growth and development.

Molybdenum (Mo) is recognized as a vital micronutrient crucial for plant development, physiological processes, and soil biology. Recent studies have extensively explored the impact of Mo on various plants, revealing significant findings regarding its effects on physiological and growth parameters. In cotton (*Gossypium* spp.), Mo plays a pivotal role in influencing crucial physiological processes that underpin productivity. Applications of Mo have produced notable effects on cotton plants, including enhancements in plant height, wet weight, root length, and both wet and dry weights (Chen et al. 2023).

The average content of molybdenum in soil ranges from 0.1 to 5.0 mg kg^{-1} soil. Generally, the heavier the soil, the more molybdenum it contains. The content of the forms of this microelement present in the soil usually does not exceed 10 per cent of its total content. Active molybdenum in soil solutions is present in very small concentrations from 10^{-8} to $8x10^{-8}$ mol dm^{-3}, mainly as MoO_4^{2-}. It forms various primary and secondary minerals in soil, is present in many organic compounds, binds to the surface of hydrated iron and aluminium oxides and silty minerals, and is soluble in soil solution (Kalembasa, Kuziemska 2013). Molybdenum (Mo), apparently an essential micronutrient for plants, plays a very important role in other biological processes in plants. Molybdenum undergoes various antagonistic processes in soil,z and its content decreases with decreasing pH and increasing temperature. In plants, it is found in enzymes involved in nitrogen fixation and nitrate reduction processes. It is also a component of enzymes (oxidases) that catalyse other processes in plants (Kabata-Pendias 2000).

Molybdenum is not biologically active. However, it is an integral part of an organic protein complex called molybdenum co-factor (Moco). Moco binds to molybdoenzymes (molybdenum-requiring enzymes) found in most higher plants (Zimmer, Mendel 1999, Kaiser et al. 2005, Mendel, Kruse 2012, Bittner 2014). An important aspect of molybdenum as a plant nutrient is its role in NO_3 reduction as a co-factor for nitrate reductase (NR) – Hamlin et al. (2007). Molybdenum application positively affects plant height, plant wet weight, root length, root wet weight and root dry weight in cotton plants by supporting basic physiological processes vital for growth and development, especially nitrogen metabolism and nutrient uptake.

Soil conditions are closely linked to microorganism activity and are considered reliable indicators of environmental stress impact on soils (Epelde et al. 2008). Due to limited studies on the effects and diversity of Mo^{2+} in soils, there is insufficient evidence to evaluate the biological implications of this metal group's stress on soil environments, thus hindering assessment of the scale of the Mo^{2+}-related issue. Maintaining optimal levels of essential micronutrients such as molybdenum is critical for sustaining healthy plant growth and maximizing crop productivity. Balancing their availability in soil and ensuring their uptake by plants in optimal amounts are essential for sustainable agriculture.

This study aimed to investigate the effects of seed, soil, and foliar applications of molybdenum on: (i) plant height, plant wet weight, plant dry weight, plant root length, and plant root dry and wet weights; (ii) plant stomatal conductance, SPAD value, and leaf area; and (iii) soil enzyme activities of cotton plants.

MATERIALS AND METHODS

Material

The study was conducted in the greenhouse facilities of the Department of Field Crops at Harran University Faculty of Agriculture. It was initiated on 9 May 2023 and concluded with harvest on 30 June 2023. The Fiona variety of cotton was used as the experimental plant material. Ammonium molybdate [$(NH_4)_6Mo_7O_{24}$] served as the source of molybdenum nutrient. The study applied two different Mo doses (25 µg Mo kg^{-1} and 50 µg Mo kg^{-1}) via three different methods: seed coating, soil application, and foliar spraying. The fertilizers used in the study include Urea (46% N) and Diammonium Phosphate (DAP, 18% N-46% P_2O_5). The application doses of DAP and Urea were determined as 200 kg ha^{-1} for each. The soil material was collected from a depth of 0-30 cm in the research and application area, sieved through a 2 mm sieve, and then filled into pots. The soil in the research area exhibits a clayey-loamy texture with a slightly alkaline pH and a highly calcareous structure. It is deficient in nitrogen and phosphorus, sufficient in potassium, and has inadequate organic matter content (Table 1).

Table 1

Some soil properties of the experimental site

Soil texture	Org matter (%)	pH	Calcareous (%)	P (kg da^{-1})	K (%)	N (%)	EC (µS cm^{-1})
Clay	1.00	8.00	28.6	1.66	143.1	0.11	140.0

Methods

The experiment

Applications

1) control: no molybdenum application
2) treatment: seed coating with 25 μg Mo kg^{-1},
3) treatment: soil application with 25 μg Mo kg^{-1},
4) treatment: foliar spraying with 25 μg Mo kg^{-1},
5) treatment: seed coating with 50 μg Mo kg^{-1},
6) treatment: soil application with 50 μg Mo kg^{-1},
7) treatment: foliar spraying with 50 μg Mo kg^{-1}.

In the study conducted according to a randomized block design with 3 replications, plastic pots were used. The pots had a height of 18 cm, a bottom diameter of 7.5 cm, and a top diameter of 12 cm. At the beginning of the experiment, no treatments were applied to the pots in the 1st, 3rd, 4th, 6th, and 7th environments, and 5 seeds were sown in each pot. For the 2nd environment (25 μg Mo kg^{-1}) and the 5th environment (50 μg Mo kg^{-1}), the seeds were soaked in Mo solution for 24 h, and sowing was carried out after 24 hours. For the other treatments, 200 ml of Mo solution from the 3rd environment (25 μg Mo kg^{-1}) and the 6th environment (50 μg Mo kg^{-1}) was applied to the soils. For foliar spraying applications, Mo solutions prepared for the 4th environment (25 μg Mo kg^{-1}) and the 7th environment (50 μg Mo kg^{-1}) were sprayed on the plant leaves 28 days after germination.

Soil and plant parameters analysed

The root and stem lengths are expressed in cm, and their fresh and dry weights are measured in grams (Acar et al. 2011). The plant's SPAD value was determined (Johnson and Saunders 2002), stomatal conductivity was measured using a leaf porometer (Decagon SC^{-1}) between 11:00 AM and 3:00 PM (Ben-Gal et al. 2009), and leaf area was calculated using the Image-J program for the leaf's surface area. Soil parameters, including soil pH and electrical conductivity (EC), were determined following the methods outlined by Jackson (1958), in addition to which catalase enzyme activity (Beck, 1971), urease enzyme activity (Hoffmann and Teicher, 1961), and dehydrogenase enzyme activity (Tabatabai, 1982) were measured.

RESULTS AND DISCUSSION

Results

The effects of different doses of Mo applied to seed, soil and plant on some physiological parameters of cotton plant are described below.

Plant height (cm)

In the study, significant differences were found among treatments and across different treatment doses for plant height, with statistical significance at (p<0.01). Compared to the control group (16.53 cm), the lowest plant height was recorded with the foliar application of 25 µg Mo kg^{-1} (17.46 cm), while the highest plant height was observed with the soil application of 50 µg Mo kg^{-1} (20.80 cm). Molybdenum applications to seeds, leaves, and soil positively influenced plant height across all treatments compared to the control group (Figure 1). Specifically, application of 25 µg Mo kg^{-1} and

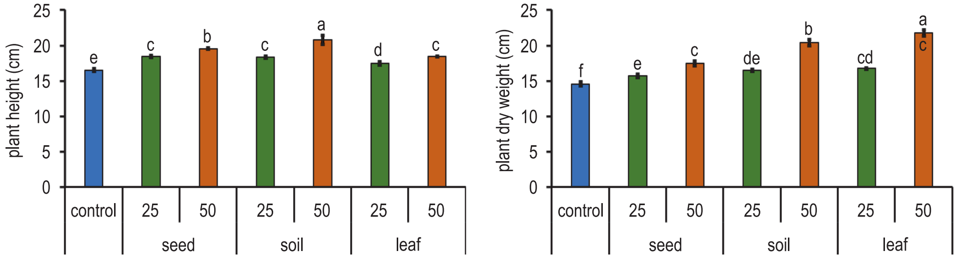

Fig. 1. Effect of Mo application on plant height and plant root length

50 µg Mo kg^{-1} to seeds, soil, and leaves increased plant height as follows: 25 µg Mo kg^{-1} seed (11.49%), 50 µg Mo kg^{-1} seed (18.33%), 25 µg Mo kg^{-1} soil (10.89%), 50 µg Mo kg^{-1} soil (25.83%), 25 µg Mo kg^{-1} leaf (5.63%), and 50 µg Mo kg^{-1} leaf (11.49%). Among the treatments, the greatest increase was observed with 50 µg Mo kg^{-1} in soil, while the smallest increase was observed with 25 µg Mo kg^{-1} in foliar treatment.

Plant root length (cm)

In the study, statistical significance (p<0.01) was found between treatments and across different treatment doses in terms of root length. The shortest root length was recorded with the seed application of 25 µg Mo kg^{-1} (34.50 cm), while the longest root length was observed with the foliar application of 50 µg Mo kg^{-1} (47.80 cm), compared to the control group (32.03 cm) – Figure 1. Among the treatments and application doses (25 µg Mo kg^{-1} and 50 µg Mo kg^{-1} applied to seeds, soil, and leaves, respectively), the greatest increases in root length were found with 25 µg Mo kg^{-1} seed (7.71%), 50 µg Mo kg^{-1} seed (19.89%), 25 µg Mo kg^{-1} soil (13.21%), 50 µg Mo kg^{-1} soil (39.96%), 25 µg Mo kg^{-1} leaf (14.98%), and 50 µg Mo kg^{-1} leaf (49.23%). The highest increase in root length due to Mo application was observed with the 50 µg Mo kg^{-1} foliar application, while the smallest increase was observed with the 25 µg Mo kg^{-1} seed application.

Plant fresh weight (g)

In the study, statistical significance (p<0.01) was found between treatments and across different treatment doses in terms of plant wet weight.

The lowest plant wet weight was recorded from the 25 µg Mo kg^{-1} application to foliar (1.05 g), while the highest plant wet weight was observed from the 50 µg Mo kg^{-1} application to soil (2.55 g) compared to the control group (0.96 g) (– Figure 2). For treatments with 25 µg Mo kg^{-1} and 50 µg Mo kg^{-1}

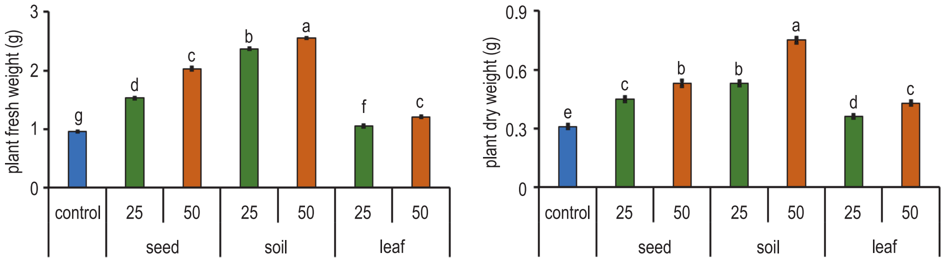

Fig. 2. Effect of Mo application on plant fresh weight and plant dry weight

applied to seeds, soil, and leaves, plant wet weight increased as follows: 25 µg Mo kg^{-1} seed (59.37%), 50 µg Mo kg^{-1} seed (111.46%), 25 µg Mo kg^{-1} soil (146.88%), 50 µg Mo kg^{-1} soil (165.62%), 25 µg Mo kg^{-1} leaf (9.38%), and 50 µg Mo kg^{-1} leaf (26.04%). Among the treatments, the greatest increase in plant wet weight due to Mo application was observed with 50 µg Mo kg^{-1} applied to soil, while the smallest increase was observed with 25 µg Mo kg^{-1} foliar application.

Plant dry weight (g)

In the study, significant differences were found between treatments and across different treatment doses in terms of plant dry weight, with statistical significance at ($p<0.01$). When comparing plant dry weight to the control group (0.31 g), the lowest dry weight was recorded with the 25 µg Mo kg^{-1} application to foliar (0.36 g), while the highest dry weight was observed with the 50 µg Mo kg^{-1} application to soil (0.75 g) – Figure 2. Among the different doses applied to seeds, soil, and leaves (25 µg Mo kg^{-1} and 50 µg Mo kg^{-1}), the highest increases in plant dry weight were observed with 25 µg Mo kg^{-1} seed (45.16%), 50 µg Mo kg^{-1} seed (70.97%), 25 µg Mo kg^{-1} soil (70.97%), 50 µg Mo kg^{-1} soil (141.94%), 25 µg Mo kg^{-1} leaf (16.13%), and 50 µg Mo kg^{-1} leaf (38.71%). The greatest increase in plant dry weight due to Mo application was found with 50 µg Mo kg^{-1} applied to soil, whereas the smallest increase was found with 25 µg Mo kg^{-1} in foliar applications.

Plant root wet weight (g)

In the study, statistically significant differences ($p<0.01$) were found between treatments and across different treatment doses in terms of root wet weight. When comparing root wet weight to the control group (0.54 g), the lowest root wet weight was recorded with the 25 µg Mo kg^{-1} application to seeds (0.64 g), while the highest root wet weight was observed with the 50 µg Mo kg^{-1} application to soil (1.15 g) – Figure 3. For treatments with 25 µg

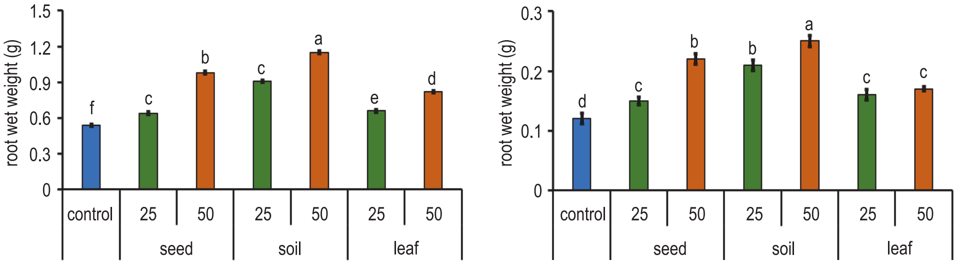

Fig. 3. Effect of Mo application on root wet and root dry weights of plants

Mo kg^{-1} and 50 μg Mo kg^{-1} applied to seeds, soil, and leaves, respectively, the highest root wet weights were obtained with 25 μg Mo kg^{-1} seed (18.52%), 50 μg Mo kg^{-1} seed (81.48%), 25 μg Mo kg^{-1} soil (68.52%), 50 μg Mo kg^{-1} soil (112.96%), 25 μg Mo kg^{-1} leaf (22.22%), and 50 μg Mo kg^{-1} leaf (51.85%). Among the treatments, the greatest increase in root wet weight due to Mo application was observed with the 50 μg Mo kg^{-1} soil application, while the smallest increase was observed with the 25 μg Mo kg^{-1} seed application.

Plant root dry weight (g)

In the study, significant differences were found among treatments and across different treatment doses in terms of root dry weight at a statistically significant level of (p<0.01). When compared with the control group (0.12 g), the lowest root dry weight was observed with the 25 μg Mo kg-1 application to seeds (0.15 g), while the highest root dry weight was observed with the 50 μg Mo kg^{-1} application to soil (0.25 g) – Figure 3. The increases in root dry weight percentages were 25% for 25 μg Mo kg^{-1} seed, 83.33% for 50 μg Mo kg^{-1} seed, 75.00% for 25 μg Mo kg^{-1} soil, 108.33% for 50 μg Mo kg^{-1} soil, 33.33% for 25 μg Mo kg^{-1} leaf, and 41.67% for 50 μg Mo kg^{-1} leaf. Among the treatments, the greatest increase with Mo application was observed with 50 μg Mo kg^{-1} soil, while the smallest increase was observed with 25 μg Mo kg^{-1} application to seeds.

Stomatal conductance

In the study, statistically significant differences (p<0.01) were found in stomatal conductance between treatments and across different treatment doses. When compared to the control group (322.36 mmol m^{-2} S^{-1}), the lowest conductance was observed with the 25 μg Mo kg^{-1} application to seed (329.66 mmol m^{-2} S^{-1}), while the highest stomatal conductance was recorded with the 50 μg Mo kg^{-1} application to soil (356.43 mmol m^{-2} S^{-1}) – Figure 4. Applications of 25 μg Mo kg^{-1} and 50 μg Mo kg^{-1} to seeds, soil, and leaves increased stomatal conductance as follows: 25 μg Mo kg^{-1} seed (2.05%), 50 μg Mo kg^{-1} seed (7.98%), 25 μg Mo kg^{-1} soil (6.74%), 50 μg Mo kg^{-1} soil (10.53%), 25 μg Mo kg^{-1} leaf (7.09%), and 50 μg Mo kg^{-1} leaf (10.25%). Among the treatments, the greatest increase in stomatal conductance due to Mo

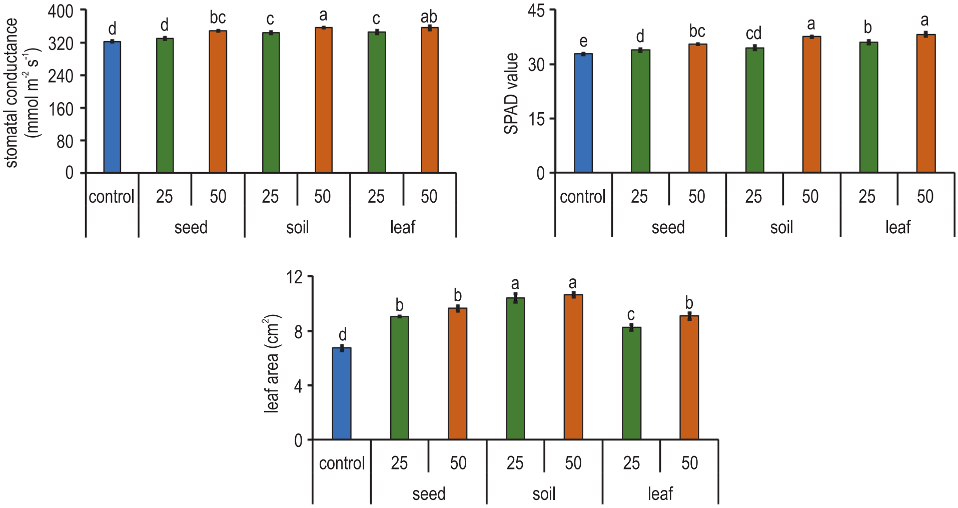

Fig. 4. Effect of Mo application on stomatal conductance, SPAD value and leaf area

application was observed with the 50 μg Mo kg^{-1} soil application, while the smallest increase was observed with the 25 μg Mo kg^{-1} seed application.

SPAD value

In the study, statistical significance (p<0.01) was observed in terms of SPAD value between treatments and across different treatment doses. When comparing SPAD values to the control group (32.80), the lowest SPAD value was recorded with the 25 μg Mo kg^{-1} application to plant seeds (33.86), while the highest value was observed with the 50 μg Mo kg^{-1} foliar application (38.16) – Figure 4. Specifically, applications of 25 μg Mo kg^{-1} and 50 μg Mo kg^{-1} to seeds, soil, and leaves increased SPAD values by 25 μg Mo kg^{-1} (3.23%), 50 μg Mo kg^{-1} (8.11%), 25 μg Mo kg^{-1} (5.18%), 50 μg Mo kg^{-1} (14.42%), 25 μg Mo kg^{-1} (9.85%), and 50 μg Mo kg^{-1} (16.34%), respectively. Among the treatments, the greatest increase in SPAD value due to Mo application was observed with the 50 μg Mo kg^{-1} foliar application, while the smallest increase was observed with the 25 μg Mo kg^{-1} seed application.

Leaf area (cm^2)

In the study, statistically significant differences (p<0.01) were observed in leaf area between treatments and across different treatment doses. When comparing leaf area to the control group (6.73 cm^2), the smallest leaf area was recorded with the 25 μg Mo kg^{-1} foliar application (8.26 cm^2), while the largest leaf area was observed with the 50 μg Mo kg^{-1} application to soil (10.64 cm^2) – Figure 4). Specifically, applications of 25 μg Mo kg^{-1} and 50 μg Mo kg^{-1} to seeds, soil, and leaves increased leaf area as follows: 25 μg Mo kg^{-1} seed (34.62%), 50 μg Mo kg^{-1} seed (43.09%), 25 μg Mo kg^{-1} soil (54.38%), 50 μg Mo kg^{-1} soil (58.10%), 25 μg Mo kg^{-1} leaf (22.73%), and 50 μg Mo kg^{-1} leaf (34.77%). Among the treatments, the greatest increase in leaf area due to Mo application was observed with the 50 μg Mo kg^{-1} soil application, while the smallest increase was observed with the 25 μg Mo kg^{-1} foliar application.

The effect of different doses of Mo applied to seed, soil and plant leaves on some parameters of soil enzymes

Catalase enzyme activity

Significant differences (p<0.01) were found between treatments and across different treatment doses in terms of catalase (CAT) enzyme activity in soils. When comparing CAT enzyme activity to the control group (6.66 ml O_2 g^{-1} dm 5 min^{-1}), the lowest activity was recorded with the application of 25 μg Mo kg^{-1} through cotton seeds (11.33 ml O_2 g^{-1} dm 5 min^{-1}), while the highest activity was observed with the application of 50 μg Mo kg^{-1} through soil (14.66 ml O_2 g^{-1} dm 5 min^{-1}) – Figure 5. Soil CAT enzyme activity showed an increase with higher application doses. Specifically, applications of 25 μg Mo kg^{-1} and 50 μg Mo kg^{-1} to seeds, soil, and leaves increased CAT activity as follows: 25 μg Mo kg^{-1} seed (70.12%), 50 μg Mo kg^{-1} seed (85.14%), 25 μg Mo kg^{-1} soil (110.21%), 50 μg Mo kg^{-1} soil (120.12%), 25 μg Mo kg^{-1} leaf (85.13%), and 50 μg Mo kg^{-1} leaf (95.19%). Among the treatments, the greatest increase in CAT activity due to Mo application was observed with the 50 μg Mo kg^{-1} application to soil, while the smallest increase was observed with the 25 μg Mo kg^{-1} application to seeds. CAT activity increased significantly in the soil treatments compared to the other applications.

Urease enzyme activity

In terms of soil urease enzyme activity, significant differences (p<0.01) were found between treatments and across different treatment doses. When comparing urease enzyme activity to the control group (5.69 μg N g^{-1} dry soil h^{-1}), the lowest activity was recorded with the application of 25 μg Mo kg^{-1} through foliar spraying (6.29 μg N g^{-1} dry soil h^{-1}), while the highest urease enzyme activity was observed with the application of 50 μg Mo kg^{-1} through soil (15.42 μg N g^{-1} dry soil h^{-1}) – Figure 5. Application of 25 μg Mo kg^{-1} and 50 μg Mo kg^{-1} to seeds, soil, and leaves resulted in the following increases in urease enzyme activity: 25 μg Mo kg^{-1} seed (45.52%), 50 μg Mo kg^{-1} seed (68.01%), 25 μg Mo kg^{-1} soil (98.24%), 50 μg Mo kg^{-1} soil (171.0%), 25 μg Mo kg^{-1} leaf (10.54%), and 50 μg Mo kg^{-1} leaf (114.59%). The greatest increase was observed with the application of 50 μg Mo kg^{-1} to soil, while the smallest increase was observed with the application of 25 μg Mo kg^{-1} through foliar spraying.

Dehydrogenase activity

Statistically significant differences were found among applications and different doses in terms of DHG in soils (p<0.01). Among DHG applications,

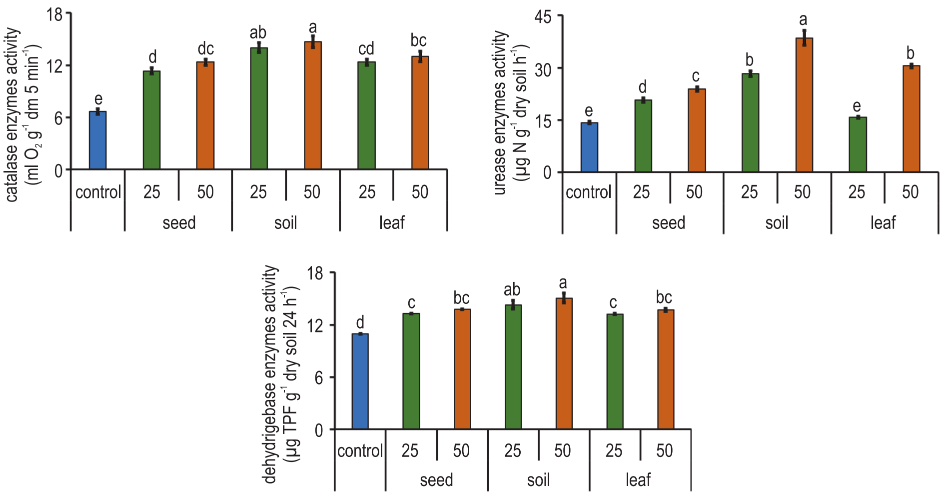

Fig.5. Effect of the Mo application on soil catalase, urease and dehydrogenase enzyme activities

the lowest DHG was observed after the application of 25 µg Mo kg^{-1} on leaves (13.24 µg TPF g^{-1} dry soil 24 h^{-1}), compared to the control group (10.93 µg TPF g^{-1} dry soil 24 h^{-1}), while the highest DHG was obtained after the application of 50 µg Mo kg^{-1} on soil (15.05 µg TPF g^{-1} dry soil 24 h^{-1}) – Figure 5. The highest increase was observed for the Mo doses applied on soil among all applications and different doses. Application of 25 µg Mo kg^{-1} and 50 µg Mo kg^{-1} to seeds, soil, and leaves resulted in increasing doses as follows: 25 µg Mo kg^{-1} seed (21.68%), 50 µg Mo kg^{-1} seed (26.26%), 25 µg Mo kg^{-1} soil (30.74%), 50 µg Mo kg^{-1} soil (37.69%), 25 µg Mo kg^{-1} leaf (21.13%), and 50 µg Mo kg^{-1} leaf (25.34%). Among applications, the highest increase due to Mo application was found at 50 µg Mo kg^{-1} applied to soil, and the least increase was observed after the application of 25 µg Mo kg^{-1} onto leaves.

Effect of Mo application on some chemical parameters of soil

Soil pH values

In the study, Mo application in different doses applied to seeds, soil, and plant leaves was found to be statistically insignificant (p<0.01) in terms of pH value among treatments and different doses. Partial or observable changes in soil pH were detected due to Mo applications on seeds, soil, and leaves, but no statistically significant effect was observed (Figure 6).

Electrical conductivity (EC)

In the study, molybdenum (Mo) applications at different doses to seeds, soil, and plants showed no statistically significant effect on electrical conductivity (EC) values among the applications or doses (p<0.01). Partial or observable changes in soil EC values resulting from seed, soil, and foliar Mo appli-

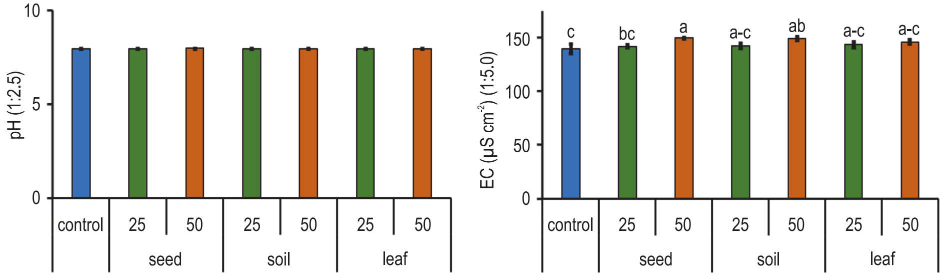

Fig. 6. Effect of Mo application on soil pH and EC

cations were detected; however, these changes were not statistically significant (Figure 6).

Principal component analysis (PCA)

Principal component analysis (PCA) was used to demonstrate relationships among numerous variables reduced to two principal components. Component 1 explains 69% of the total variability and is strongly associated with such variables as plant fresh weight, plant dry weight, plant root dry weight, plant root fresh weight, and plant height. These variables are represented by arrows extending prominently to the right in the graph, indicating a strong positive relationship with Component 1. On the other hand, variables like pH and EC show a negative trend in Component 1, indicating an inverse relationship with this component (Fig 7).

Component 2 explains 11% of the total variability and is particularly associated with variables such as SPAD and plant root length, as indicated by arrows extending upwards in the graph, suggesting a strong positive relationship with component 2. Variables like stomatal conductivity and catalase enzyme activity show a negative trend in component 2 (Figure 7).

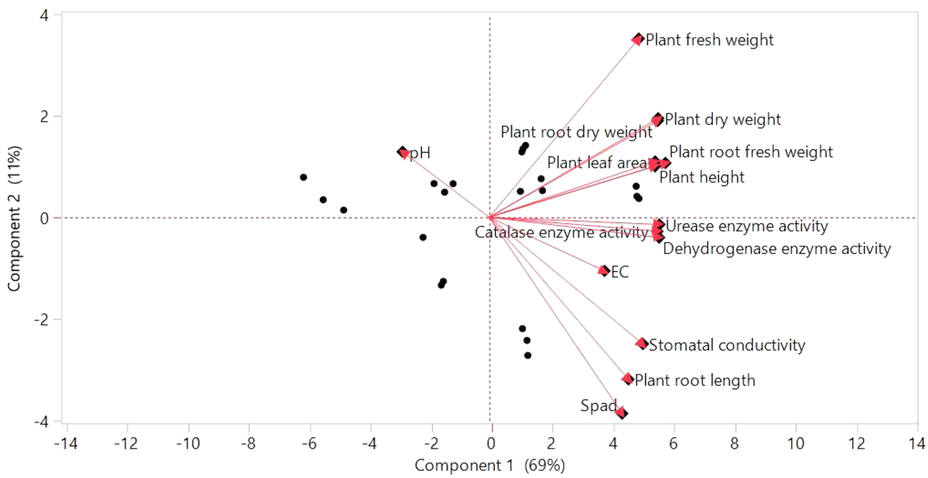

Fig. 7. Principal component analysis (PCA) of the examined parameters

Variables such as plant fresh weight, plant dry weight, plant root dry weight, plant root fresh weight, and plant height are aligned in the same direction and closely positioned to each other in the graph, indicating positive correlations among these variables. pH and EC variables, however, are closely aligned but extend towards the opposite direction of component 1, suggesting a positive correlation with each other but an inverse relationship with the other variables. SPAD and plant root length variables show an upward trend in component 2, indicating a positive relationship with component 2 and potentially weaker relationships with the other variables (Figure 7).

DISCUSSION

Molybdenum (Mo) is a fundamental micronutrient for plants (Bortels 1930, Arnon, Stout 1939), playing a significant role in various physiological processes, plant development, and soil biological functions, including nitrogen fixation, nitrate reduction, and enzymatic activities (Bittner 2014). Molybdenum plays a crucial role in the growth and development of cotton plants (*Gossypium* spp.) and affects various physiological processes that are crucial for their productivity.

The effect of molybdenum (Mo) application on the physiological and growth parameters of various plants (soybean, cotton, tobacco, etc.) has been extensively studied in recent years (Nasar et al. 2022, Oliveira et al. 2022). In the current study, Mo was applied at different levels to the seeds, soil, and leaves of cotton plants and tested collectively. Observations revealed that increasing Mo doses consistently led to a statistically significant (p<0.01) increase in plant height, plant fresh weight, root length, root fresh weight, and root dry weight (Oliveira et al. 2022). This effect can be attributed to Mo-mediated enhancement of photosynthetic efficiency (Gao et al. 2010, Missio et al. 2023). The observed increase in total plant biomass in this study is also consistent with findings reported by other researchers (Raza et al. 2020, Hussain et al. 2021, Henrique et al. 2024), and can be linked to the role of Mo in nitrogen (N) metabolism, its ability to improve N use efficiency, and its promotion of plant growth (Probst et al. 2021). These effects are likely influenced by water availability during the plant's growth period, which enhances Mo solubilization and uptake by the plant (Jiang et al. 2020).

The application of Mo to soil ensures a consistently higher concentration of molybdenum in the roots, which is necessary for the stability and activity of nitrate reductase enzyme. This condition indicates that the plant part may be more active in nitrogen assimilation. Although we did not determine the activity of this enzyme directly, the high urease and DHG enzyme activities in the soil after molybdenum applications may have resulted from applying

50 μg Mo kg^{-1} to soil (Kovács et al. 2015). The most effective outcome was achieved by increasing doses of Mo applied to the soil. According to Mendel and HaÈnsch (2002), applying Mo to soil facilitates continuous molybdenum supply to the root zone, thereby enhancing optimal growth conditions. Similarly, the positive correlation between various morphological plant traits and Mo application aligns with previous studies (Heshmat et al. 2021, Nasar et al. 2022), suggesting that applications of Mo enhanced various enzymatic processes related to plant growth and development (Bittner 2014, Probst et al. 2021, Ru et al. 2023).

In the study, the effect of Mo applications on SPAD values was found to be statistically significant (p<0.01) among treatments and different doses. The lowest SPAD value among treatments was observed after the application of 25 μg Mo kg^{-1} to plant seeds compared to the control group, while the highest value was obtained after the application of 50 μg Mo kg^{-1} to leaves. Application of Mo to leaves increased the plant's SPAD value by 16.34%. The increase in the SPAD value observed in this study could be linked to the role of Mo in the formation of the Mo cofactor involved in the synthesis of chlorophyll and other essential biomolecules, as suggested by several researchers (Schwarz et al. 2009, Mendel 2013, Li et al. 2018, Probst et al. 2021, Bavaresco et al. 2022). This highlights the importance of Mo as a micronutrient for plants and its significant role in nitrogen metabolism (Walkley, Black 1934). Application of Mo to soil is believed to increase nitrogen accumulation, seed yield, and seed protein content in plants (Campo et al. 2009). Similar results have been reported previously by Kaiser et al. (2005) and Kadlag et al. (2006).

In the study, significant differences (p<0.01) were found among treatments and different doses of Mo in terms of stomatal conductivity, SPAD value, and leaf area. The plant's stomatal conductivity increased with increasing doses of Mo applied. When the highest dose of molybdenum was applied to seeds, the plant's stomatal conductivity increased by 7.98%. This can be attributed to Mo assisting in the early development of nitrogen enzymes dependent on the plant (Bavaresco et al. 2022, Cheng et al. 2022). Urease and DHG in the soil are crucial for nitrogen assimilation, which affects stomatal conductivity. This increase in stomatal conductivity can result from enhanced nitrogen uptake and assimilation processes.

Applying 50 μg Mo kg^{-1} via leaves increased stomatal conductivity by 10.53%, marking the highest stomatal conductivity measured at this dose. This indicates that molybdenum is directly transported to leaves, where it can quickly enter the plant's metabolic processes. Molybdenum applied to leaves can swiftly address deficiencies and enhance the activity of molybdenum-dependent enzymes in the leaves (Jiang et al. 2007, Yoon et al. 2019). Application of molybdenum via leaves can result in rapid adjustments of stomatal conductivity, thereby improving the plant's water use efficiency and overall physiological performance (Henrique et al. 2024).

Stomatal conductivity is a metric that reflects the ability of leaf stomata to facilitate gas exchange with the environment. Stomata, located on the leaf epidermis, respond promptly to environmental fluctuations (Henry et al. 2019). The highest stomatal conductivity was achieved with the application of 50 μg Mo kg^{-1} via leaves, increasing stomatal conductivity by 10.79%. Molybdenum applied to soil becomes available to the plant through root uptake (Ahmed et al. 2020). Soil-applied molybdenum facilitates the synthesis of enzymes involved in various metabolic pathways, including those related to nitrogen and carbon metabolism. These enzymes indirectly influence stomatal conductivity by regulating photosynthesis and transpiration rates. Optimal levels of molybdenum in the soil ensure proper functioning of these enzymes, promoting balanced stomatal conductivity by maintaining adequate CO_2 uptake and regulating water loss (Grassi et al. 2002, Sepehri, Sanavy 2003, Zangani et al. 2021, Henrique et al. 2024). Therefore, soil application of Mo has shown better results compared to seed and foliar applications.

The leaf area of the plant showed statistically significant differences among treatments and different doses at (p<0.01) level. Compared to the control group, the lowest leaf area was observed after the application of 25 μg Mo kg^{-1} to seeds, while the highest leaf area was achieved with the application of 50 μg Mo kg^{-1} to the soil. When molybdenum was applied to cotton seeds, it promoted early seedling vigour and root development, which can enhance nutrient uptake and overall plant growth, contributing to a broader leaf area during the plant's growth stages (Kandil et al. 2013, Jamali et al. 2023). Additionally, molybdenum aids nitrogen metabolism essential for chlorophyll synthesis and photosynthesis, thereby increasing photosynthetic efficiency and leading to a plant growing larger and healthier leaves (Imran et al. 2019, Zangani et al. 2021)

Applying molybdenum to leaves via spraying can directly address any deficiencies and provide optimal nutrient levels for leaf development. Foliar application allows molybdenum to be quickly absorbed by the plant, rapidly correcting deficiencies and promoting leaf growth. Larger leaves can capture more sunlight and perform more photosynthesis, ultimately contributing to an increase in leaf area. Soil application of molybdenum indirectly influences leaf area by enhancing nutrient availability (Heshmat et al. 2021). Molybdenum facilitates nitrogen fixation by nitrogen-fixing bacteria, thereby increasing nitrogen availability in the soil. Adequate nitrogen levels in the soil promote vigorous vegetative growth, leading to larger leaves and an overall increase in leaf area.

Application of molybdenum (Mo) to soil, seeds, and leaves can deeply influence the activities of key enzymes in the soil such as urease, dehydrogenase, and catalase, each playing significant roles in soil health and nutrient cycling. Urease catalyzes the hydrolysis of urea into ammonia and carbon dioxide, a crucial step in nitrogen mineralization (Tabatabai, Bremner, 1972). In the current study, the lowest urease activity was observed after the application of 25 μg Mo kg^{-1} to leaves, while the highest urease enzyme activity was obtained with the application of 50 μg Mo kg^{-1} to soil. This indicates that molybdenum enhances its role as a cofactor for urease in soil (Schwarz et al. 2009). When applied to soil, molybdenum can increase urease activity, facilitating the breakdown of urea into ammonium, a nitrogen form readily used by plants (Sakin et al. 2024). Adequate molybdenum levels promote efficient nitrogen use, supporting plant growth and development. Conversely, molybdenum deficiency can limit urease activity, reducing nitrogen availability for plants and potentially leading to nutrient deficiencies (Kalembasa, Kuziemska 2013).

There were statistically significant differences (p<0.01) among treatments and different doses of molybdenum (Mo) applications in terms of soil dehydrogenase (DHG) activity. Among the DHG activities observed, the lowest DHG was recorded after the application of 25 μg Mo kg^{-1} to leaves compared to the control group, while the highest DHG was obtained with the application of 50 μg Mo kg^{-1} to soil. Dehydrogenase enzymes play a role in redox reactions, organic matter decomposition, and are crucial in nutrient cycling (Ekenler, Tabatabai 2002). Molybdenum indirectly affects dehydrogenase activity by promoting the growth and activity of molybdenum-dependent soil microorganisms, including nitrogen-fixing bacteria. Therefore, the increased dehydrogenase activity stimulated by molybdenum application to soil accelerates organic matter decomposition, releases nutrients for plant uptake, and enhances soil fertility. However, excessive levels of molybdenum can disrupt microbial communities and decrease dehydrogenase activity, thereby affecting soil health (Dick 2015, Sakin et al. 2024).

The catalase enzyme activity of soils showed statistically significant differences among different application doses (p<0.01). Among the applications, the lowest catalase activity was observed in the treatment with 25 μg Mo kg^{-1} applied to cotton seeds, while the highest activity was obtained from the treatment with 50 μg Mo kg^{-1} applied to the soil. Catalase enzymes decompose hydrogen peroxide into water and oxygen, reducing oxidative stress, which is crucial for maintaining soil and plant health. Molybdenum is necessary for catalase synthesis and function (Nematpour, Eshghizadeh 2024). Application of molybdenum can enhance catalase activity in soil, thereby increasing its resistance to oxidative stress. The elevated catalase activity facilitated by molybdenum helps protect plant roots and soil microorganisms from oxidative damage, promoting healthier soil conditions and enhanced plant growth.

Adequate levels of molybdenum in the soil can enhance healthy plant growth, root exudation, and organic matter decomposition, which releases weak organic acids that can gradually lower soil pH. This decrease has been observed in soil samples but at very low rates, potentially mitigated by soil buffering capacity that limits significant fluctuations in soil pH. Moreover, molybdenum's role in nitrogen metabolism can indirectly influence soil electrical conductivity (EC) by affecting nutrient availability and uptake by plants, thereby impacting nutrient cycling and ion concentrations in soil solution. However, the extent of these indirect effects depends on various factors including soil type, molybdenum application doses, and interactions with other soil components. Therefore, while molybdenum does not directly alter soil pH and EC, it can partially modify them, with its influence on plant and microbial processes indirectly affecting these soil characteristics (Kabata-Pendias 2000).

CONCLUSIONS

This study highlights the essential role of molybdenum (Mo) in supporting plant growth, development, and soil enzyme activities. Particularly in calcareous soils, Mo emerges as a vital element that facilitates nitrogen metabolism, soil fertility, and enzymatic functions. The research revealed significant increases in various growth parameters and soil enzyme activities following Mo applications. Consistent increases were observed across all application trials in plant height, root length, fresh and dry weights, stomatal conductance, SPAD value, and leaf area. The highest improvement in these parameters was achieved with the soil application of 50 µg kg^{-1} Mo, underscoring the efficacy of soil-based Mo supplementation in enhancing plant vigour and productivity.

Additionally, soil enzyme activities, including urease, dehydrogenase and catalase, were significantly enhanced by Mo applications. This underscores a critical role of Mo in soil functions, such as nutrient cycling and organic matter decomposition. The highest increases in soil enzyme activities were also recorded with the soil application of 50 µg kg^{-1} Mo, reaffirming its effectiveness in boosting soil health and enzymatic activity.

The findings clearly demonstrate the superiority of soil application of Mo over foliar and seed treatments. The results strongly advocate for the inclusion of Mo in soil management practices to optimize plant growth, development, and soil fertility.

In conclusion, this study provides valuable insights into the effectiveness of Mo applications in supporting plant growth and soil enzymatic functions. Future research and practical applications aimed at maximizing the benefits of Mo supplementation hold great potential for sustainable agriculture and ecosystem health.

Author contributıons

A.Ç. – conceptualization, data curation, formal analysis; S.C – funding acquisition, investigation, methodology; V.B. – resources, software, supervision; H.Y. – validation; Z.F. – visualization, writing the original draft; E.S.

– writing – review and editing of manuscript. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interests

The authors declare that there are no conflicts of interest related to this article.

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