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Sesame seed Treatment with Molybdenum and Zinc to Improve Tolerance to Water Deficit

Tratamento de Sementes de Gergelim com Molibdênio e Zinco para Tolerância ao Déficit Hídrico

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Abstract

Sesame cultivation during the second cropping season in the Brazilian Cerrado is highly exposed to periods of water deficit, particularly during early plant development. Seed treatment with micronutrients may enhance crop establishment and mitigate the effects of abiotic stress. This study aimed to evaluate the effect of sesame seed treatment with zinc (Zn) and molybdenum (Mo) on physiological seed quality and early plant development under water deficit conditions. The experiment was conducted in two phases: laboratory assays in a completely randomized 4 × 4 design, with doses of 0, 0.5, 1.0, and 1.5 g kg^{-1} of Zn and Mo; and a greenhouse trial in a 4 × 2 factorial scheme (micronutrient treatments × water availability). Germination, seedling emergence, shoot dry mass, and root dry mass were evaluated. The dose of 0.5 g kg^{-1} of Zn and Mo promoted superior germination performance and greater initial biomass accumulation, whereas higher doses reduced paper-test germination, indicating possible phytotoxic effects. In the greenhouse, water deficit significantly reduced plant growth. Seed treatment with 0.5 g kg^{-1} Mo, alone or combined with Zn, enhanced root development, even under stress conditions. It is concluded that seed treatment with 0.5 g kg^{-1} Mo, with or without Zn, improves early sesame development, although it does not confer effective tolerance to severe short-term water deficit.

Keywords: *Sesamum indicum* L. Micronutrients. Germination. Biomass. Abiotic stress.

Resumo

O cultivo do gergelim em segunda safra no Cerrado brasileiro apresenta elevada exposição a períodos

de déficit hídrico, especialmente na fase inicial de desenvolvimento. O tratamento de sementes com micronutrientes pode favorecer o estabelecimento inicial da cultura e mitigar efeitos do estresse abiótico. Objetivou-se avaliar o efeito do tratamento de sementes de gergelim com zinco (Zn) e molibdênio (Mo) sobre a qualidade fisiológica e o desenvolvimento inicial das plantas sob déficit hídrico. O experimento foi conduzido em duas fases: ensaios laboratoriais, em delineamento inteiramente casualizado (4 × 4), com doses de 0; 0,5; 1,0 e 1,5 g kg^{-1} de Zn e Mo; e ensaio em casa de vegetação, em esquema fatorial 4 × 2 (tratamentos com micronutrientes × disponibilidade hídrica). Avaliaram-se germinação, emergência, massa seca da parte aérea e do sistema radicular. Doses de 0,5 g kg^{-1} de Zn e Mo promoveram melhor desempenho germinativo e maior acúmulo de biomassa inicial, enquanto doses superiores reduziram a germinação em papel, indicando possível efeito fitotóxico. Em casa de vegetação, o déficit hídrico reduziu significativamente o crescimento das plantas. O tratamento com 0,5 g kg^{-1} de Mo, isoladamente ou associado ao Zn, favoreceu o desenvolvimento radicular, inclusive sob estresse. Conclui-se que o tratamento de sementes com 0,5 g kg^{-1} de Mo, com ou sem Zn, melhora o desenvolvimento inicial do gergelim, embora não confira tolerância efetiva ao déficit hídrico severo de curta duração.

Palavras-chave: *Sesamum indicum* L. Micronutrientes. Germinação. Biomassa. Estresse Abiótico.

1 Introduction

The cultivated area of sesame (Sesamum indicum L.) in Brazil has grown continuously each year, with the 2024/2025 harvest estimated at 608 thousand hectares and a total production of 399.4 thousand metric tons (Conab, 2025). Due to the short life cycle of this crop (90 to 110 days), nearly all of this acreage is located in the Brazilian Cerrado region, cultivated as a second crop season immediately following soybean harvest. Because of the timing of the second cropping season, these crops are generally more vulnerable to dry spells and potential periods of water deficit during the growing cycle, particularly under current climate instability. Furthermore, second-crop sowing occurs immediately after soybean harvest, when soil moisture is frequently sub-optimal, thereby compromising early crop establishment.

Given the yield losses caused by abiotic stress, products containing macro- and micronutrients have been developed to enhance plant tolerance to adverse conditions, particularly to alleviate the adverse effects of low water availability. Among the nutrients included in such formulations to stimulate early plant growth are zinc (Zn) and molybdenum (Mo) (Souza *et al*., 2022).

Zinc participates in tryptophan biosynthesis reactions - a precursor of indole-3-acetic acid (IAA) molecules - which plays a pivotal physiological role in cell division and seedling growth, participating in seed germination and early seedling development, as well as offering protection against soil-borne pathogens and conferring enhanced tolerance to abiotic stresses (Cakmak, 2005; Taiz *et al*., 2017; Yadav *et al*., 2020).

Molybdenum is the micronutrient required in the smallest quantities by plants; nevertheless, it is essential because its function is directly tied to nitrogen (N) metabolism, serving as a co-factor for critical enzymes such as nitrate reductase, xanthine dehydrogenase, aldehyde oxidase, and sulfite

oxidase. Plant Mo deficiency results in reduced leaf chlorophyll concentration, lower photosynthetic efficiency, and disruption of N metabolism, leading to nitrate (NO3-) accumulation in plant tissues (Borkert, 1989; Souza *et al.,* 2022).

Seed treatment to supply micronutrients represents a practical approach to delivering essential nutrition for early field establishment, and studies have shown promising results in crops such as maize and soybean (Freitas *et al.*, 2020; Carmo-Filho *et al*., 2025). For sesame, few studies in the literature have yielded promising yet conclusive results (Parsaie *et al.*, 2020; Pink *et al.,* 2020). The limited information currently available does not yet guarantee the safe application of micronutrient seed treatments in small-seeded species such as sesame, particularly regarding optimal dosage, post-treatment seed physiological quality, and subsequent effects on early crop development and root establishment.

Therefore, the objective of this study was to evaluate the effect of sesame seed treatment with Zn and Mo on seed germination, early seedling development, and plant tolerance to water deficit.

2 Material and Methods

The project was conducted in two sequential phases: laboratory seed quality evaluations were carried out at the Seed Analysis Laboratory (Department of Plant Science and Phytosanitary Protection), and the greenhouse experiment was conducted at the School Farm, both belonging to the Center for Agricultural Sciences at the State University of Maranhão (UEMA), São Luís, Maranhão State, Brazil.

Phase 1: Evaluation of physiological quality of sesame seeds treated with Zn and Mo

Seeds were treated with different doses of Zn (0, 0.5, 1.0, and 1.5 g kg^{-1} of seeds) and Mo (0, 0.5, 1.0, and 1.5 g kg^{-1} of seeds) in a 4×4 factorial scheme arranged in a completely randomized design (CRD) with four replications. Sodium molybdate (Na2MoO4.2H2O) served as the Mo source, and zinc sulfate (ZnSO4) was used as the Zn source. Seeds of the sesame cultivar 'BRS Anahi' were used. For seed treatment, an aqueous solution equivalent to 1% of the seed mass was applied, and the seeds were manually agitated for 5 minutes to ensure uniform coverage.

To evaluate seed physiological quality, germination tests and sand emergence tests were performed. The germination test was conducted with four replications of 50 seeds distributed on germination paper substrate moistened with distilled water at a volume equivalent to 2.5 times the dry paper weight, placed inside transparent plastic boxes (gerbox), and incubated in a germination chamber kept at 25 °C for 6 days (Brasil, 2009). Results were expressed as the percentage of normal seedlings. First count of germination was recorded at 3 days, and final count at 6 days after sowing.

Seedling emergence was evaluated using 200 seeds per treatment (four replications of 50 seeds).

Seeds were sown in sand boxes following the standard top-of-sand procedure. First emergence count was taken at 3 days, and final emergence of normal seedlings was recorded at 6 days after sowing, with results expressed as percentage. Following the emergence count, 10 representative seedlings per replication were separated to determine shoot dry mass and root dry mass. Seedlings were dried in a forced-air oven at 60 °C for 72 hours until constant weight and weighed on an analytical balance, with results expressed in mg seedling^{-1} (Nakagawa, 1999).

Phase 2: Evaluation of early plant growth and response to water deficit

Based on the physiological quality parameters evaluated in Phase 1, the two best-performing doses of Mo and Zn were selected to evaluate initial plant growth and water deficit response. This second experiment was conducted in a greenhouse using a 4×2 factorial scheme (4 micronutrient combinations × 2 water regimes) in a randomized complete block design (RCBD) with four replications. The treatments were:

T1: 0.0 g kg^{-1} Mo + 0.0 g kg^{-1} Zn (Control)

T2: 0.5 g kg^{-1} Mo + 0.0 g kg^{-1} Zn

T3: 0.0 g kg^{-1} Mo + 0.5 g kg^{-1} Zn

T4: 0.5 g kg^{-1} Mo + 0.5 g kg^{-1} Zn

Sesame was sown in 8-liter pots filled with a soil substrate mixture composed of Latosol, sand, and commercial substrate in a 1:1:1 volumetric ratio. Five seeds were sown per pot, and seedlings were thinned to two plants per pot after establishment. The experiment was conducted up to 35 days after emergence (DAE).

Irrigation was managed gravimetrically by weighing the pots every 3 days and adding water to restore soil moisture to field capacity, which had been previously determined. For water deficit treatments, irrigation was completely withheld for 7 consecutive days, starting at 20 DAE (from 20 to 27 DAE). Planting fertilization was performed according to regional technical recommendations for sesame. At 35 DAE, shoot dry mass (g) and root dry mass (g) were evaluated by drying plant parts in a forced-air oven at 60 °C for 72 hours until constant weight and weighing on an analytical balance (Nakagawa, 1999).

Data from both phases were subjected to Analysis of Variance (ANOVA), and mean comparisons were performed using Sisvar software (Ferreira, 2003). Due to the nature of the research, ethics committee approval was not required by Embrapa or UEMA.

3 Results and Discussion

The summary of Analysis of Variance (ANOVA) for germination paper tests and sand emergence tests of sesame seeds treated with varying doses of Mo and Zn is presented in Table 1.

Table 1 - Analysis of variance (ANOVA) for laboratory trials of sesame seeds treated with different doses of Zn and Mo. Tabela 1. Análise de variância dos ensaios em laboratório com sementes de gergelim tratadas com diferentes doses de Zn e Mo

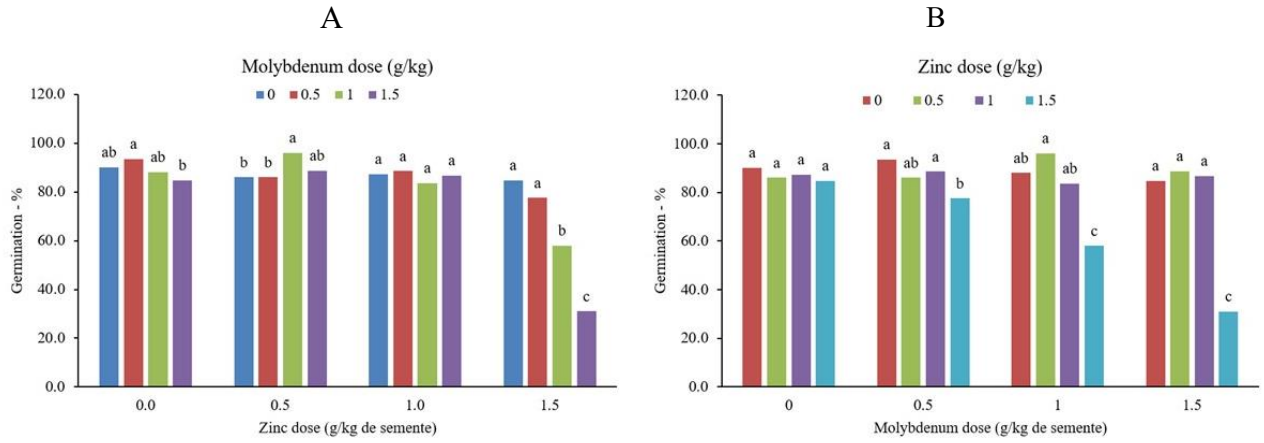
Source of Variation	Germination %	Emergence %	Shoot Dry Mass (g)	Root Dry Mass (g)
	Pr>Fc			
Zn	0.000**	0.055^{ns}	0.000**	0.000**
Mo	0.000**	0.366 ^{ns}	0.000**	0.776 ^{ns}
Zn x Mo	0.000**	0.619 ^{ns}	0.004**	0.413 ^{ns}

* Significant at $p < 0.05$; **Significant at $p < 0.01$ by F-test; ^{ns} Non-significant.
Source: research data.

Significant effects ($p < 0.01$) of micronutrient seed treatments were observed for paper germination percentage, shoot dry mass, and root dry mass, whereas sand seedling emergence showed no significant main effects or interaction (Table 1). A significant interaction ($p < 0.01$) between Zn and Mo doses was detected for paper germination percentage and shoot dry mass.

The average paper germination percentages across treatments at 6 days after sowing are shown in Figure 1.

Figure 1 - Mean germination percentage of sesame seeds treated with different doses of Mo and Zn in a paper substrate test evaluated six days after sowing. (A) Sliced effect of Mo doses within Zn doses; (B) Sliced effect of Zn doses within Mo doses



*Means followed by the same lowercase letter within each dose level do not differ significantly ($p > 0.05$).
Source: research data.

The observed reduction in seed germination at doses exceeding 1.0 g kg^{-1} Mo and 1.5 g kg^{-1} Zn (Figure 1A, B) aligns with recent evidence demonstrating that excessive micronutrient application can cause ionic imbalance, oxidative stress, and loss of membrane integrity during seed imbibition, particularly in small-seeded species such as sesame. In Zn-treated seeds, elevated concentrations can impair membrane reorganization and trigger excessive reactive oxygen species (ROS) production, negatively affecting germination (Broadley *et al.*, 2019; Hassan *et al.*, 2020). Similarly, although required in trace amounts, excessive Mo application exerts toxic effects that disrupt early seedling

metabolic processes (Wu et al., 2020).

Mean emergence percentages in the sand substrate evaluated at 6 DAE are presented in Table 2.

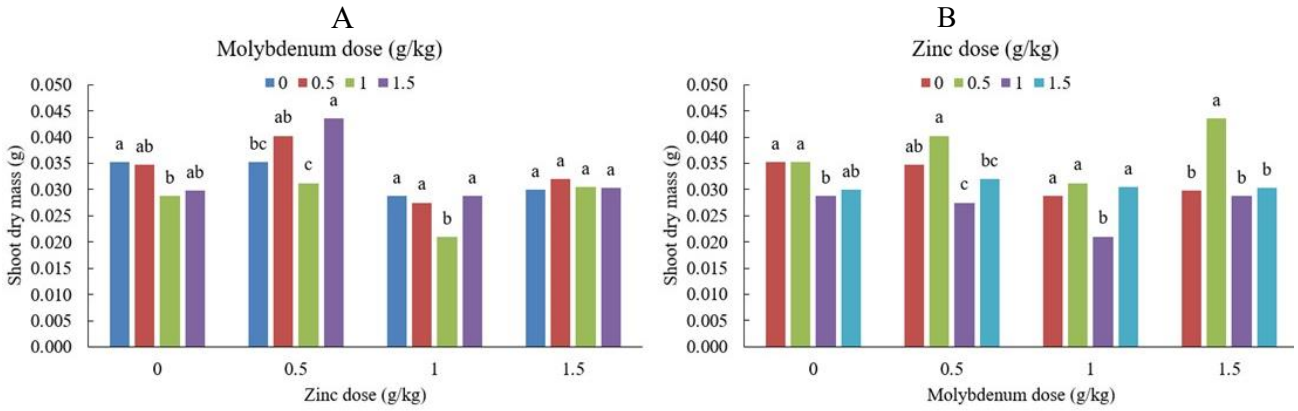
Table 2 - Mean seedling emergence percentages of sesame seeds treated with different doses of Mo and Zn in a sand substrate test evaluated six days after sowing

Mo Dose (g kg⁻¹ of seeds)	Emergence (%) – Sand test				
	Zn dose (g kg⁻¹ of seeds)				Mean
	0.0	0.5	1.0	1.5	
0	88.0	89.5	89.0	88.5	88.8a
0.5	88.5	84.5	94.0	85.0	88.0a
1	94.0	86.0	94.0	88.0	90.5a
1.5	91.0	91.0	92.5	89.5	91.0a
Mean	90.4a	87.8a	92.4a	87.8a	89.6

*Means followed by the same lowercase letter in rows or columns do not differ significantly ($p > 0.05$).
Source: research data.

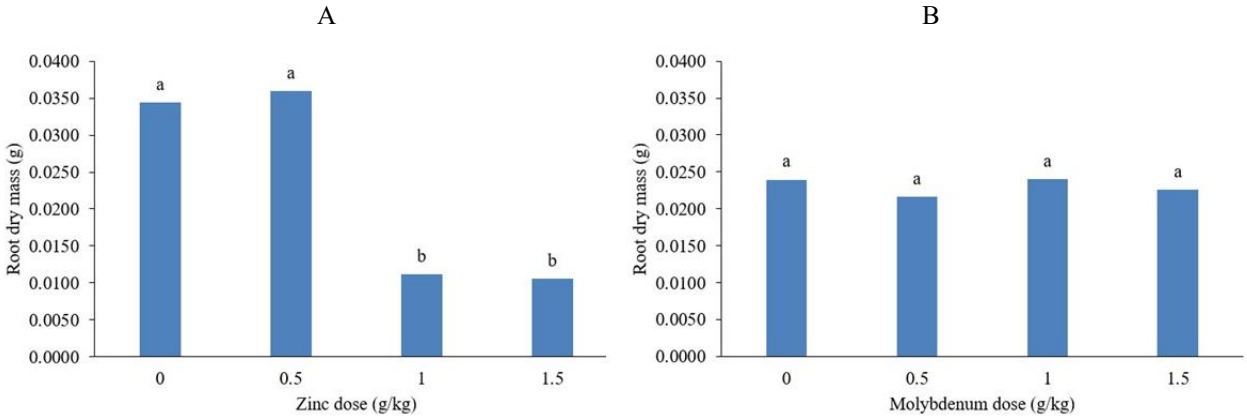
The absence of significant differences in the sand emergence test (Table 2) suggests that the sand substrate attenuated potential osmotic or phytotoxic effects observed on paper, likely due to greater ion dilution and adsorption within the matrix. Similar findings were reported by Parsaie et al. (2020), who noted that micronutrient enrichment in sesame seeds enhanced vigor under controlled conditions, but responses varied depending on the germination substrate medium. Figures 2 and 3 display mean values of shoot dry mass and root dry mass of seedlings harvested 6 days after sowing in sand.

Figure 2 - Mean shoot dry mass of sesame seedlings treated with different doses of Mo and Zn evaluated six days after sowing in sand. (A) Sliced effect of Mo doses within Zn doses; (B) Sliced effect of Zn doses within Mo doses



* Means followed by the same lowercase letter within each set do not differ significantly ($p > 0.05$).
Source: research data.

Figure 3 - Mean root dry mass of sesame seedlings treated with different doses of Zn (A) and Mo (B) evaluated six days after sowing in sand.



* Means followed by the same lowercase letter do not differ significantly ($p > 0.05$).
Source: research data.

For shoot dry mass (Figure 2), a significant interaction was found between Mo and Zn doses. Sliced analysis indicated that optimal responses were generally achieved with 0.0 and 0.5 g kg-¹ doses for both micronutrients.

For root dry mass (Figure 3), Mo doses had no significant effect, whereas Zn doses showed a significant influence ($p < 0.01$\$), with maximum root dry weight observed at 0.0 and 0.5 g kg-¹ Zn.

The physiological role of Zn as a key structural and functional cofactor in multiple enzymes - along with its role in tryptophan biosynthesis and auxins (IAA) - explains the enhanced early development at moderate doses (Hafeez *et al*., 2020).

Increased auxin production promotes cellular division and elongation, resulting in higher early biomass accumulation.

Regarding root growth, the positive effect of lower Zn doses supports recent findings indicating that Zn seed treatment stimulates root architecture by increasing lateral root length and density (Rehman *et al*., 2015; Wu *et al*., 2020).

Conversely, the lack of significant root response to Mo during the laboratory stage suggests that its primary influence is linked to nitrogen metabolism in later developmental stages.

Based on these results, doses of 0.0 and 0.5 g kg^{-1} of Mo and Zn were selected for the greenhouse experiment evaluating plant performance under full irrigation versus a 7-day water deficit applied at 20 DAE.

Table 3 summarizes the ANOVA for greenhouse parameters evaluating the effects of water regime and micronutrient seed treatments on early sesame growth.

Table 3 - Summary of ANOVA for greenhouse trial of sesame plants from seeds treated with Zn and Mo under full irrigation and a 7 day water deficit (20 to 27 DAE)

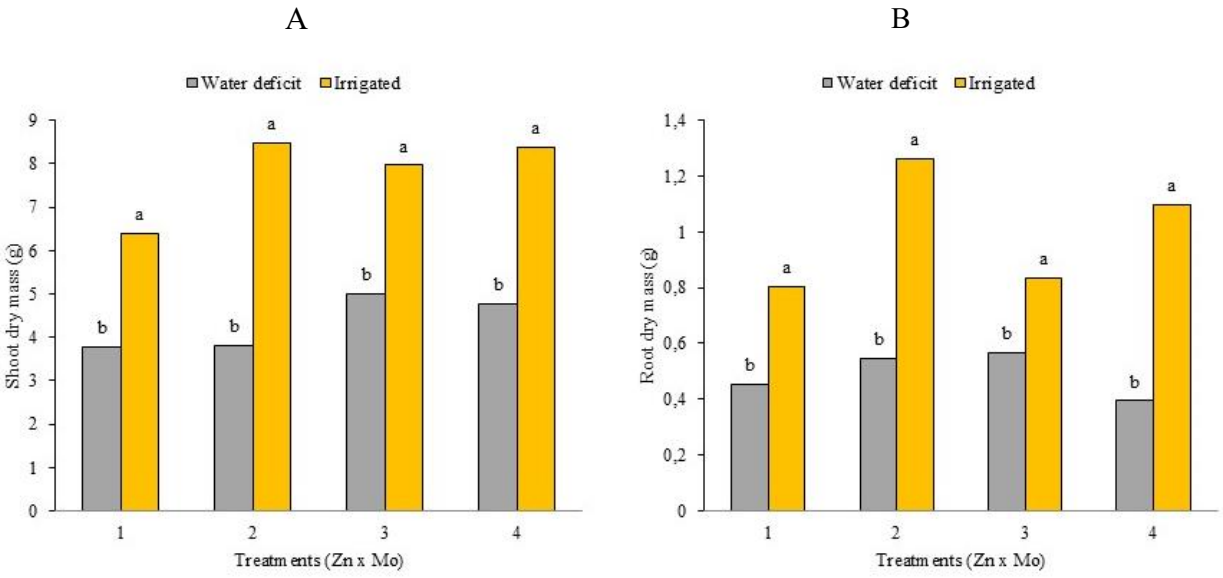
Sources of Variation		Shoot Dry Mass	Root Dry Mass
	DF	Pr>Fc	
Micronutrients (Zn e Mo)	3	0.090^{ns}	0.018*
Irrigation regime	1	0.000**	0.000**
Block	3	0.102^{ns}	0.108^{ns}
Micro x Irrig	3	0.378^{ns}	0.016*
Error	21		
Total	31		

* Significant at $p < 0.05$; **Significant at $p < 0.01$ by F-test; ns Non-significant.
Source: Prepared by the authors (2026).

Water availability significantly influenced ($p < 0.01$) both shoot dry mass and root dry mass, confirming the high vulnerability of sesame to early-stage water restriction (Table 3). Furthermore, a significant interaction ($p < 0.05$) between micronutrient treatments and water regime was observed for root dry mass.

To examine how seed treatment influenced growth under each water regime, sliced interaction analysis was conducted (Figure 4A, B).

Figure 4 - Mean shoot dry mass (A) and root dry mass (B) of sesame plants at 35 DAE originating from seeds treated with different doses of Mo and Zn (T1: 0.0 Mo and 0.0 Zn; T2: 0.5 Mo and 0.0 Zn; T3: 0.0 Mo and 0.5 Zn; T4: 0.5 Mo and 0.5 Zn) under full irrigation versus 7 day water deficit (20–27 DAE)



Means followed by the same lowercase letter within each treatment do not differ significantly by Tukey's test ($p > 0.05$).
Source: research data.

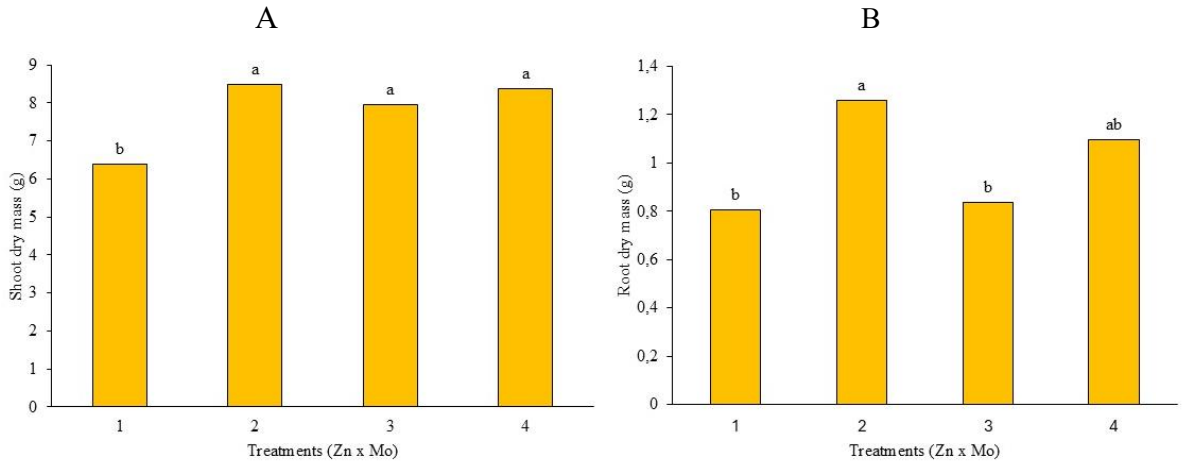
In the greenhouse trial, water deficit caused marked reductions in growth across all seed treatments (Table 3, Figure 4). Sliced comparison within each seed treatment showed that plants under

full irrigation achieved significantly higher biomass than those subjected to water deficit, demonstrating that micronutrient seed treatments at the tested doses did not confer complete physiological immunity to short-term severe water stress.

The reduction in shoot and root biomass under water limitation is consistent with reports showing that water restriction impairs cell expansion, photosynthetic activity, and biomass accumulation in oilseed crops (Farooq *et al*., 2017; Haghpanah *et al*., 2024). However, under water deficit conditions, plants derived from micronutrient-treated seeds showed superior root growth performance compared to the untreated control (T1). This indicates that while seed treatment does not fully eliminate severe stress damage, it promotes early root development, which may enhance soil volume exploitation under field conditions.

To further detail the micronutrient treatment effects under specific water regimes, sliced treatment comparisons within full irrigation (Figure 5) and water deficit (Figure 6) were conducted.

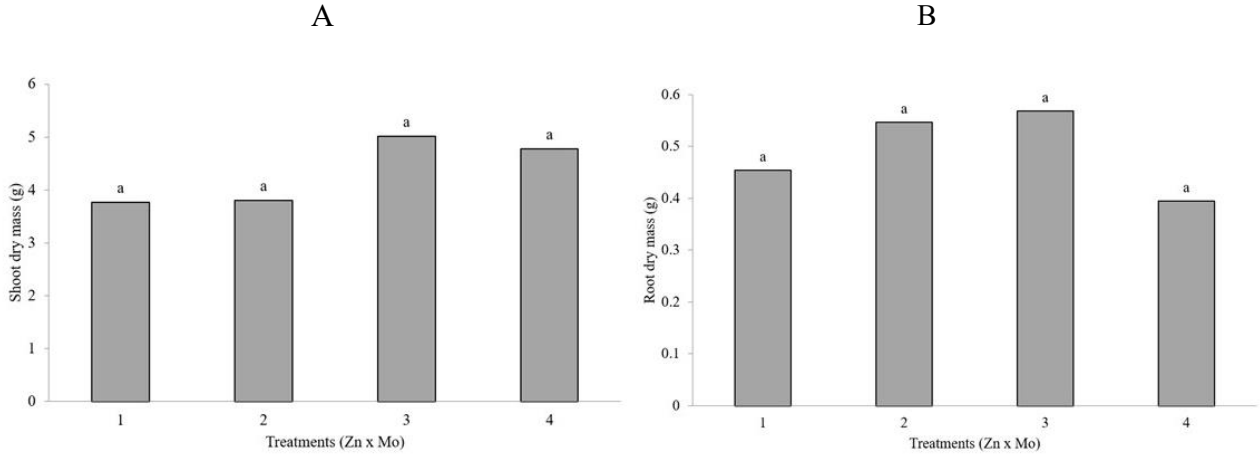
Figure 5 - Mean shoot dry mass (A) and root dry mass (B) of sesame plants at 35 DAE under full irrigation conditions across seed treatments



Means followed by the same lowercase letter do not differ significantly by Tukey's test ($p > 0.05$).
Source: research data.

Under full irrigation (Figure 5), seed treatment with 0.5 g kg^{-1} Mo alone (T2) resulted in the highest shoot dry mass and significantly greater root dry mass compared to the control (T1) and Zn alone (T3). This demonstrates that under non-limiting water conditions, seed treatment with Mo effectively stimulates early vegetative growth in sesame. The superior performance of 0.5 g kg^{-1} Mo (T2), alone or combined with Zn (T4), highlights the role of Mo as an indispensable cofactor of nitrate reductase, a key enzyme in N assimilation. Proper nitrogen metabolism is crucial for protein and chlorophyll synthesis, promoting shoot and root growth (Wu *et al*., 2020). Adequate Mo supply via seed treatment has been reported to boost nitrogen use efficiency and early seedling vigor (Kovacs *et al.*, 2015; Souza *et al*., 2022).

Figure 6 - Mean shoot dry mass (A) and root dry mass (B) of sesame plants at 35 DAE under 7 day water deficit conditions across seed treatments



Means followed by the same lowercase letter do not differ significantly by Tukey's test ($p > 0.05$).
Source: research data.

Under water deficit conditions (Figure 6), treatments T2, T3, and T4 consistently exhibited higher numerical biomass and improved root growth relative to the control (T1). Particularly, T2 (0.5 g kg^{-1} Mo) promoted robust root development, which can contribute to drought avoidance in subsequent growth stages by facilitating deeper soil exploration. Recent studies emphasize that delivering micronutrients via seed treatment offers advantages such as high nutrient use efficiency, reduced application rates, and minimal soil fixation losses (Dimkpa; Bindraban, 2016).

Nevertheless, responses depend heavily on dosage, species, and environmental context, underscoring the importance of establishing safe operational ranges. As noted by Afzal et al. (2020), elements like Mo are required in trace quantities, and excessive doses lead to toxicity; however, appropriate seed-applied doses minimize nutrient losses and support initial crop establishment, as corroborated by the present study.

4 Conclusion

Sesame seed treatment with zinc and molybdenum influences seed physiological quality and early plant development in a dose-dependent manner. Doses of 0.5 g kg^{-1} of seeds for both micronutrients promoted superior germination and early biomass accumulation, whereas higher doses showed phytotoxic effects in paper germination tests.

Under greenhouse conditions, water deficit significantly reduced plant growth regardless of seed treatment. However, seed treatment with 0.5 g kg^{-1} Mo, alone or in combination with Zn, stimulated early plant growth, particularly root system development, under both well-watered and water-restricted conditions.

Although seed treatment with Zn and Mo did not confer complete tolerance to severe, short-term water deficit, it offers potential benefits for initial crop establishment. Further field research is

recommended to evaluate whether these early physiological gains translate into improved crop yields under rainfed conditions exposed to mid-season dry spells.

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