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Molybdenum's role in enhancing cold stress tolerance of rocket plants

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ABSTRACT

Plants are exposed to various abiotic stress factors throughout their life cycles. Among these, low temperature is a significant abiotic stressor in agriculture that can substantially impact plant growth, development, yield, and crop quality. This study aims to investigate the effects of molybdenum (Mo) application on the 'Bengi' rocket variety under conditions of low-temperature stress. The research was conducted in climate-controlled conditions at Siirt University. The 'Bengi' rocket variety was utilized as the plant material, and a growing medium composed of a 2:1 (2v:1v) mixture of peat and perlite was employed. The experimental design followed a randomized complete block design with three replications, each consisting of ten plants. The treatment groups included: control, 25 ppm Mo, 50 ppm Mo, 75 ppm Mo, 12 h of cold stress, 24 h of cold stress, 12 h of cold stress + 25 ppm Mo, 12 h of cold stress + 50 ppm Mo, 12 h of cold stress + 75 ppm Mo, 24 h of cold stress + 25 ppm Mo, 24 h of cold stress + 50 ppm Mo, and 24 h of cold stress + 75 ppm Mo. The control group was maintained under a 16/8 h light-dark cycle at temperatures of 20°C during the day and 17°C at night. Seedlings were subjected to cold stress at 4°C for 12 h, followed by normal conditions at 20°C for an additional 12 h. At the conclusion of the study, various parameters were evaluated, including plant height, stem diameter, leaf number, fresh weight, dry weight, moisture content, SPAD value, ion leakage, turgor loss, and relative water content. The results revealed that the highest plant height (20.47 cm) was recorded with the 75 ppm Mo treatment, while the lowest height (13.50 cm) was observed under the 24-h cold stress + 75 ppm Mo treatment. The maximum stem diameter (2.71 mm) was also noted with the 75 ppm Mo application, whereas the minimum diameter (2.15 mm) occurred with the 12-h cold stress + 75 ppm Mo treatment. The leaf count was highest at 9.667 leaves with the 75 ppm Mo treatment and lowest at 6.00 leaves with the 12-h cold stress + 75 ppm Mo treatment. The SPAD value reached its peak at 47.97 with the 75 ppm Mo application, while the lowest value (38.13) was recorded under the 12-h cold stress condition. Fresh weight was highest at 10.22 g with the 25 ppm Mo treatment and lowest at 4.88 g with the 50 ppm Mo treatment. Additionally, the maximum moisture content was found to be 93% with the 25 ppm Mo application, while the minimum was 85% with the 50 ppm Mo treatment. In conclusion, this study demonstrates that molybdenum applications exert complex effects on plant growth, which vary according to Mo concentration and the duration of cold stress. These findings provide a crucial basis for developing optimal molybdenum usage and plant protection strategies against cold stress in agricultural practices.

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1. Introduction

The increasing environmental issues have led to plants being more frequently exposed to abiotic stresses such as extreme temperatures, high salinity, and dehydration, which in turn restrict plant growth and crop productivity (He et al., 2018; Waqas et al., 2019). To survive under these stressful conditions, plants must develop appropriate defense responses. In this context, signaling molecules, including various protein kinases, protein phosphatases, transcription factors, and other regulatory proteins, play critical roles in different stress signaling pathways to maintain homeostasis (Zhu, 2002; Ding et al., 2020). The transmission of these signals leads to the upregulation or downregulation of target genes (Tuteja, 2007; Ding et al., 2020), allowing plants to adapt their physiological and morphological states (Volkov, 2015; Liu et al., 2018).

Low-temperature stress significantly influences the cold adaptation of plants in temperate climates. There are two distinct types of stress: chilling (0-15°C) and freezing (below 0°C), which are crucial for subtropical plants. To adapt to these conditions, plants have developed the ability to perceive and transmit cold signals. In response to low-temperature stress, the C-repeat (CRT) binding factor/dehydration response element binding protein 1 (CBF/DREB1)-dependent signaling pathway is activated as a primary regulatory mechanism (Thomashow, 1999). Recent studies have indicated that kinases, transcription factors, and regulatory proteins involved in this mechanism lead to the activation of cold-regulated (COR) genes and increased cold tolerance (Shi et al., 2018; Ding et al., 2020). Extreme low or high temperatures are among the most significant environmental factors affecting crop species' growth and quality. Low temperatures can considerably reduce the rate of various metabolic processes, leading to crop losses (Hussain et al., 2018). Warm-season plants are particularly sensitive to temperature declines during germination and reproductive stages, resulting in injuries when exposed to chilling temperatures (Sanghera et al., 2011). Cold stress is a crucial factor determining the geographical distribution of many vegetable species and can affect yield productivity (Cao et al., 2019). Cold stress induces ion leakage in plant tissues, leading to water loss and fresh weight reduction (Lyons, 1973; Ruelland and Collin, 2012). Additionally, cold stress adversely affects physiological and metabolic processes in plants, potentially reducing enzyme activity and affecting the functionality of biochemical reactions (Balabusta et al., 2016; Cao et al., 2019).

Molybdenum (Mo) is an essential micronutrient for both plants and animals (Rana et al., 2020a; Ismael et al., 2018). Molybdenum can enhance plant resistance to stress conditions. By promoting the production of certain antioxidant enzymes, molybdenum may increase plant tolerance to environmental stresses such as water stress, temperature fluctuations, and pest attacks. Additionally, it can contribute to increased resistance against various diseases. However, many soils worldwide are adversely affected by Mo deficiency (Rana et al., 2020b; Imran et al., 2019). This deficiency is a significant agricultural problem that leads to quality and yield losses in crop species (Liu, 2001; Liu et al., 2000). The availability of molybdenum is influenced by soil pH and the concentration of organic components. In alkaline soils, molybdenum becomes more soluble and accessible to plants, while it decreases in acidic soils (Reddy et al., 1997). Molybdenum deficiency results in the development of various phenotypes that negatively impact plant growth, often associated with reduced activity of enzymes such as nitrate reductase (NR). Plants deficient in Mo typically exhibit poor growth, low chlorophyll content, and diminished ascorbic acid levels (Liu, 2002b). Soluble MoO_4 can enhance soil solubility by forming complexes with organic matter (Kaiser et al., 2005). Molybdenum deficiency manifests particularly pronounced symptoms in the Brassicaceae family, where young plants may exhibit mottling, pitting, and discoloration of leaves (Hewitt and Bolle-Jones, 1952). Furthermore, low Mo levels in the presence of nitrate fertilizers can lead to necrotic areas on leaf margins (Chatterjee and Nautiyal, 2001; Kaiser et al., 2005). Molybdenum is known to enhance the efficient utilization of nitrogen in plants, resulting in positive effects on growth, development, productivity, and stress tolerance. However, both molybdenum deficiency and excess can cause physiological imbalances in plants.

Rocket (*Eruca sativa*), a member of the Brassicaceae family, is recognized as a cool-season vegetable. While its exact origin is not definitively established, it is believed to have originated from Mediterranean countries (Bianco and Boari, 1996). The optimal temperature range for rocket cultivation is identified as 15-20°C. Temperatures below 10°C adversely affect seed germination and plant development. When suitable growing conditions are met, the time from sowing to harvest maturity is shortened in summer.

Low-temperature conditions can lead to an increase in the quantity of aromatic compounds, which may also cause deterioration in plant quality. In the absence of extreme low or high temperatures, and with adequate soil and air moisture during spring and autumn, rocket exhibits good plant development and results in high-quality produce (Eşiyok et al., 1998). Due to its positive health effects, the consumption of green leafy vegetables, including rocket, is increasing. The plant's appetite-stimulating properties and high nutritional value contribute to its growing significance. Additionally, rocket has been reported to possess aphrodisiac properties and is utilized in the treatment of certain diseases, the healing of insect bites, and in diet meals (Eşiyok, 1996).

This study investigated the effects of various treatments on the 'Bengi' rocket variety, including control, 25 ppm Mo, 50 ppm Mo, 75 ppm Mo, 12 h of cold stress, 24 h of cold stress, 12 h of cold stress + 25 ppm Mo, 12 h of cold stress + 50 ppm Mo, 12 h of cold stress + 75 ppm Mo, 24 h of cold stress + 25 ppm Mo, 24 h of cold stress + 50 ppm Mo, and 24 h of cold stress + 75 ppm Mo. The parameters assessed included plant height, stem diameter, leaf number, fresh weight, dry weight, moisture content, SPAD value, ion leakage, turgor loss, and relative water content. Additionally, the cold stress tolerance of the 'Bengi' rocket variety was determined.

2. Material and methods

2.1. Plant material

The 'Bengi' rocket variety was utilized in this study. This variety is characterized by broad, long leaves with a dark green color and an oval leaf structure that becomes serrated towards the lower parts. It is cultivated in spring and autumn and is known for its aromatic flavor, primarily consumed fresh. 'Bengi', being a commercial variety, was sourced from a commercial seed company.

2.2. Experimental design

The experiment was conducted in the climate chamber of the Department of Field Crops at Siirt University, as well as in a refrigerator designed for plant cultivation. The growing medium for the seedlings was prepared in a ratio of 2:1 (peat). The prepared seedling medium was placed into 45-cell trays, each with an inner depth of 5 cm and external dimensions of 6 cm, measuring 33 cm x 54 cm, before the seeds were sown.

2.3. Application of waterlogging stress

Seed sowing and seedling cultivation were conducted in a 2:1 peat-perlite medium. Once the seeds germinated and the plants reached the 3-4 leaf stage, the treatments commenced. The applications included different Mo concentrations (0, 25, 50, and 75 ppm) and cold stress durations (12 and 24 h).The treatment groups included 0 ppm, 25 ppm, 50 ppm, and 75 ppm Mo, 12-h cold stress, 24-h cold stress, 0 ppm Mo + 12-h cold stress, 25 ppm Mo + 12-h cold stress, 50 ppm Mo + 12-h cold stress, 75 ppm Mo + 12-h cold stress, 0 ppm Mo + 24-h cold stress, 25 ppm Mo + 24-h cold stress, 50 ppm Mo + 24-h cold stress, and 75 ppm Mo + 24-h cold stress. The control group was maintained under conditions of 16 h of light and 8 h of darkness, with temperatures set at 20°C during the day and 17°C at night. The first cold stress treatment involved exposing the seedlings to 4°C for 12 h, followed by a second treatment lasting 24 h. The experiment was designed with three replications, each containing 10 plants.

Hoagland's nutrient solution was used as the nutrient source for the plants. Irrigation was applied so that the drainage rate was approximately 24±2% (Schröder and Lieth, 2002). Irrigation duration was established based on careful observations and assessments conducted on the plants. The molybdenum treatments (0 ppm, 25 ppm, 50 ppm, and 75 ppm) were applied in spray form for three consecutive days. Control plants were sprayed with distilled water before being subjected to cold stress for 12 and 24 h. Following these treatments, measurements and observations were made on rocket plants on the 28th day. The parameters evaluated included plant height, stem diameter, leaf number, fresh weight, dry weight, moisture content, SPAD value, ion leakage, turgor loss, and relative water content. 2.4. Parameters investigated in experiment

2.4.1. Plant height (cm)

The height of the plant was measured from the root collar to the growing tip using a ruler (Dere et al., 2019).

2.4.2. Stem diameter (mm)

The stem diameter of the plants was measured in millimeters at the midpoint using calipers (Dere et al., 2019).

2.4.3. Leaf number (number plant^{-1})

At the end of the treatments, the number of leaves on each plant was counted and recorded (Yılmaz, 2020).

2.4.4. Fresh weight (g)

The fresh weights of the green plant samples collected at the end of the treatments were recorded using a precision balance (Yılmaz, 2020; Altuntaş et al., 2020).

2.4.5. Dry weight (g)

The fresh-weighted plants were placed in paper bags and dried in an oven at 75 °C, after which the dry weight was measured in grams (Yılmaz, 2020; Altuntaş et al., 2020).

2.4.6. Moisture content (%)

The moisture content was determined using the fresh and dry weights of the plants according to the following formula (Yasemin Koksal et al., 2016):

$$MC_{wb} = (FW - DW\ FW^{-1}) \times 100 \quad (1)$$

Where:

MCwb: Moisture content (%),

FW: Fresh weight of the plant (g),

DW: Dry weight of the plant (g).

2.4.7. Ion leakage (%)

For this purpose, 1 cm diameter leaf discs were placed in deionized water for 5 h, after which the electrical conductivity (EC1) was measured. The same discs were then placed at 75 °C for 24 h, and the conductivity of the solution was measured again (EC2). Ion leakage was calculated using the formula (Arora et al., 1998; Yasemin et al., 2017):

$$\text{Ion Leakage} = (EC2\ EC1^{-1}) \times 100 \quad (2)$$

2.4.8. Relative water content (RWC)(%)

At the end of the treatments, 1.0 cm diameter leaf discs of the same age and size were taken from each treatment. The initial fresh weights (FW) of these discs were recorded using a precision balance. The discs were then placed in Petri dishes containing distilled water for 4.0 h, after which their turgor weights (TW) were determined. The turgor-weighted samples were dried in an oven set at 70 °C for 24 h, and their dry weights (DW) were measured (Yasemin, 2020).

$$RWC(\%) = [(FW - DW)\ (TW - DW)^{-1}] \times 100 \quad (3)$$

Where:

FW: Fresh weight,

TW: Turgor weight,

DW: Dry weight.

2.4.9. Turgor loss (%)

Turgor loss was calculated based on the fresh weight and turgor weight of the leaf discs.

$$TL(\%) = [TW - FW\ TW^{-1}] \times 100 \quad (4)$$

Where:

FW: Fresh weight,

TW: Turgor weight.

2.4.10. Chlorophyll content (SPAD)

At the end of the treatments, SPAD readings were taken in triplicate for samples of the same age and size using a SPAD 502 meter (Minolta/Japan) (Daşgan et al., 2010; Dere, 2019).

2.4.11. Statistical analyses

The data obtained were subjected to variance analysis using the JUMP 7.0 software package and means that showed significant differences were grouped using the LSD multiple comparison test (Jump, 2007).

3. Results and discussion

3.1. Plant height

The effects of the treatments on plant height in rocket were found to be statistically significant (Figure 1; $p<0.001$). Among the treatments, the application of 25 ppm Mo resulted in a slight increase in plant height compared to the control group (17.37cm). Conversely, the application of 50 ppm Mo led to a decrease in plant height compared to the control group (15.83 cm). The 75 ppm Mo treatment, however, resulted in the highest increase in plant height (20.47 cm). The effects of cold stress were notable, as the 12-h cold stress application caused a significant reduction in plant height (15.33 cm). The 24-h cold stress application resulted in an even more pronounced decrease (14.83 cm). When Mo was added in conjunction with cold stress, the 12-h cold stress + 25 ppm Mo treatment showed slight increases in plant height compared to the control group (17.70 cm). In contrast, the 12-h cold stress + 50 ppm Mo treatment resulted in a drop in plant height to 16.87 cm. Notably, in the case of the 12-h cold stress + 75 ppm Mo treatment, there was an increase observed (16.90 cm) compared to the 12-h cold stress alone. For the 24-h cold stress application (14.83 cm), both the 24-h cold stress + 25 ppm Mo and the 24-h cold stress + 50 ppm Mo treatments showed slight increases in plant height (14.87 cm and 14.90 cm, respectively). However, the 24-h cold stress + 75 ppm Mo treatment led to a significant decrease in height (13.50 cm) compared to the 24-h cold stress alone.

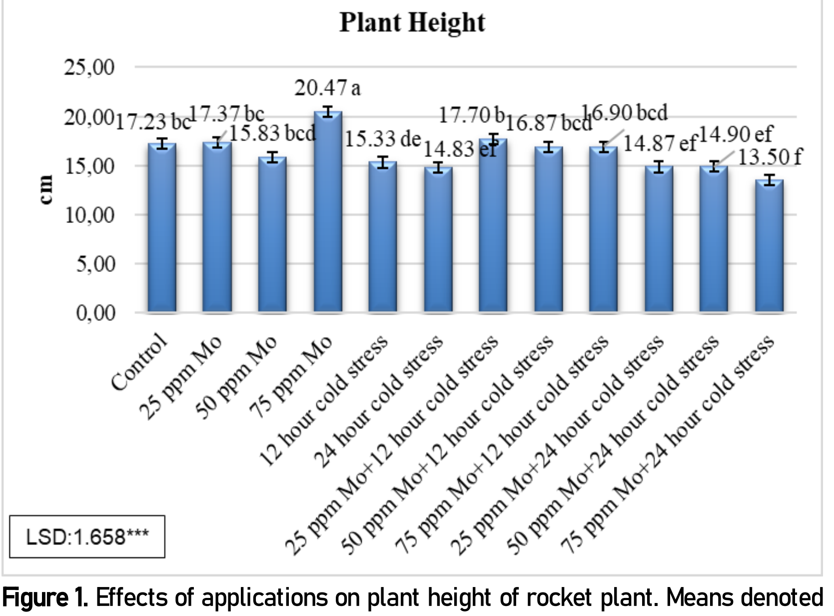

Figure 1. Effects of applications on plant height of rocket plant. Means denoted by different lowercase in letters the same column indicate significant difference ($p<0.05$) among treatments

These findings suggest that molybdenum at low concentrations (25 ppm) can slightly enhance plant growth, while higher concentrations (50 ppm) may exhibit inhibitory effects. The application of 75 ppm Mo appears to promote significant growth compared to the control group. When examining the combined effects of cold stress and molybdenum on plant height, it is observed that lower concentrations (25 ppm Mo) can yield positive effects in some instances, but higher concentrations (50 ppm and 75 ppm Mo) do not mitigate the adverse impacts of cold stress. This evaluation enhances our understanding of the effects of molybdenum on plant growth and the contribution of cold stress to these effects. The data provides a crucial starting point for determining optimal molybdenum concentrations and understanding how to protect plants against cold stress.

Our results are largely consistent with the existing literature, but they also present some important differences and new findings. It has been reported that the height of broad bean plants varies between 40.88 cm and 53.88 cm. In the case of the 'Eresen 87' variety, which typically ranges from 90 to 107 cm in height depending on the region, the observed plant height of 53.88 cm is attributed to plant stress. Although the highest plant height was recorded in the treatment with 0.05 ppm molybdenum, no statistically significant difference was found among the various treatments (Vuralın and Müftüoğlu, 2012). Furthermore, Kaiser et al. (2005) and Kovács et al. (2015) highlighted that molybdenum deficiency leads to impaired nitrogen metabolism, increased nitrate accumulation, and stunted growth, and that molybdenum application mitigates these adverse effects, thereby enhancing crop yield. This aligns with our findings, as the application of 25 ppm Mo resulted in an increase in plant height, supporting these positive effects. Zou et al. (2008) noted that molybdenum fertilization can increase yields in crops like wheat, which is also reflected in our study, where 25 ppm Mo promoted plant growth. However, the literature does not commonly report that higher concentrations of molybdenum (50 ppm and 75 ppm) consistently lead to increased yields. In our study, we observed that higher concentrations of molybdenum, particularly under cold stress, had inhibitory effects and did not mitigate the negative impact of cold stress—in fact, they often exacerbated it. This finding extends beyond the general view in the literature, suggesting that there is an optimal molybdenum concentration that has a significant impact on plant health and yield. Therefore, the effects of molybdenum vary depending on concentration and environmental stress factors, indicating the need for a more cautious and tailored approach to molybdenum fertilization in agricultural practices.

3.2. Stem diameter

The effects of the treatments on stem diameter in rocket were statistically significant (Figure 2; $p<0.05$). Among the treatments, the application of 25 ppm Mo resulted in a slight decrease in stem diameter compared to the control group (2.55 mm), while the 50 ppm Mo treatment showed a diameter similar to the control group (2.65 mm). The 75 ppm Mo treatment exhibited a slight increase in stem diameter (2.71 mm). At low concentrations (25 ppm), molybdenum caused a reduction in stem diameter, while a slight increase was observed at higher concentrations (75 ppm). The control group experienced a notable reduction in stem diameter under 12-h cold stress (2.25 mm) and 24-h cold stress (2.24 mm), indicating that cold stress has a negative impact on stem diameter, with the effect becoming more pronounced over time. In comparison to the 12-h cold stress (2.25 mm), the applications of 12-h cold stress + 25 ppm Mo and 12-h cold stress + 50 ppm Mo resulted in increases in stem diameter (2.50 mm and 2.38 mm, respectively). However, the 12-h cold stress + 75 ppm Mo treatment showed a decrease (2.15 mm). For the 24-h cold stress (2.24 mm), the 24-h cold stress + 25 ppm Mo and 24-h cold stress + 50 ppm Mo treatments resulted in increases in stem diameter (2.55 mm and 2.37 mm, respectively), while the 24-h cold stress + 75 ppm Mo treatment led to a decrease (2.24 mm). The study indicates that the application of 25 ppm Mo resulted in a slight reduction in stem diameter compared to the control group (2.55 mm), which may be attributed to the plant's inability to optimally utilize molybdenum at this concentration. The 50 ppm Mo application exhibited a diameter similar to that of the control group (2.65 mm), suggesting that molybdenum at this concentration does not significantly affect plant growth. Conversely, the 75 ppm Mo treatment demonstrated a slight increase in diameter (2.707 mm), indicating that higher concentrations of molybdenum can promote growth. Regarding the cold stress applications, the 12-h cold stress treatment caused a significant reduction in stem diameter (2.25 mm), clearly showing that cold stress negatively impacts plant growth. Similarly, the 24-h cold stress application resulted in a reduction in stem diameter (2.24 mm), with the effects of prolonged cold stress being more pronounced than those of shorter durations.

When considering the combinations of cold stress and molybdenum, the application of 25 ppm Mo along with 12-h cold stress led to a slight decrease in stem diameter (2.50 mm), indicating that molybdenum did not mitigate the adverse effects of cold stress. The 12-h cold stress + 50 ppm Mo treatment resulted in a significant decrease compared to the control group (2.38 mm), suggesting that higher molybdenum concentrations may exacerbate the negative impacts of cold stress. The 12-h cold stress + 75 ppm Mo treatment showed the most substantial reduction (2.15 mm), further demonstrating that high molybdenum concentrations can intensify the effects of cold stress. The 24-h cold stress + 25 ppm Mo treatment also resulted in a slight decrease in stem diameter (2.55 mm), indicating that this combination did not sufficiently alleviate the effects of cold stress. Likewise, the 24-h cold stress + 50 ppm Mo and 24-h cold stress + 75 ppm Mo treatments also caused reductions in stem diameter (2.37 mm and 2.24 mm, respectively). These findings highlight the complex interactions between molybdenum concentrations and cold stress on plant growth. While low concentrations of molybdenum may enhance growth slightly in certain instances, high concentrations, particularly when combined with cold stress, can lead to adverse effects. These results provide a crucial foundation for optimizing the use of molybdenum in agricultural practices and developing strategies to protect plants against cold stress.

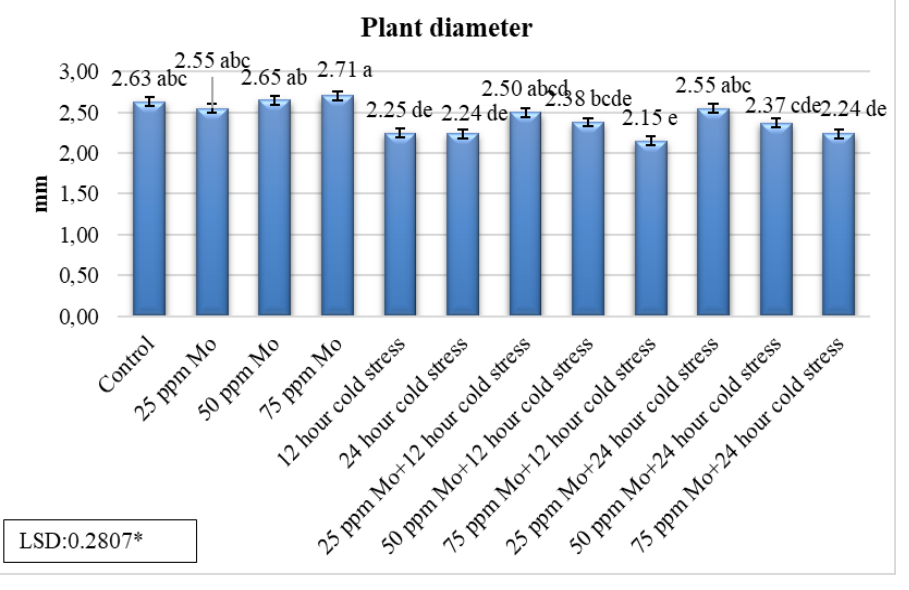

Figure 2. Effects of applications on plant diameter of rocket plant. Means denoted by different lowercase in letters the same column indicate significant difference (p<0.05) among treatments

3.3. Leaf number

The effects of the treatments on leaf number in rocket were statistically significant (Figure 3; p<0.05). No significant changes in leaf count were observed between the control group and the 25 ppm Mo treatment (8.00 leaves). However, there was a slight increase in leaf number with the 50 ppm Mo treatment (8.67 leaves), and a significant increase was noted with the 75 ppm Mo treatment (9.67 leaves). This indicates that increasing concentrations of molybdenum have a positive effect on leaf number. In the cold stress applications, a significant reduction in leaf count was observed after 12 h of cold stress (6.67 leaves), while no significant change occurred after 24 h of cold stress (8.00 leaves). The negative effects of cold stress on the plants were particularly pronounced in the short-term application. An increase in leaf number was observed in the 12-h cold stress + 25 ppm Mo treatment (8.67 leaves). In contrast, there were no changes in leaf number with the 12-h cold stress + 50 ppm Mo and 24-h cold stress + 25 ppm Mo treatments compared to the control group (8.00 leaves each). However, reductions in leaf count were observed in the 12-h cold stress + 75 ppm Mo (6.00 leaves) and 24-h cold stress + 50 ppm Mo treatments (7.33 leaves). Interestingly, a slight increase in leaf count was observed in the 24-h cold stress + 75 ppm Mo treatment (8.33 leaves).

These findings highlight the effects of molybdenum on leaf number and the contribution of cold stress to these effects. Higher concentrations of molybdenum appear to enhance leaf number, but cold stress can negatively impact this response. These results enhance our understanding of the complex interactions among factors influencing plant growth. Syaifudin et al. (2023) investigated the effects of MoK (molybdenum potassium) and NMoK (nitrate molybdenum potassium) treatments on plant growth, focusing on dry weight and yield. Their results showed that MoK treatments significantly increased the dry weight of leaves and spikes compared to the control. The NMoK treatment had a significantly higher dry weight for roots, stems, and leaves, and it also resulted in higher yields, number of grains per pot, and harvest index than the control. In conclusion, both studies highlight the beneficial effects of molybdenum on plant growth, but these effects are observed in different growth parameters. While our study focused specifically on leaf number and the interaction with cold stress, the study by Syaifudin et al. (2023) emphasized the improvement in plant biomass, yield, and other growth factors with molybdenum treatments. It was reported that the branch number values of broad bean plants varied between 3.00 and 4.75, the highest number of branches were formed in the application without molybdenum, but there was no statistically significant difference between the other applications (Vuralın and Müftüoğlu, 2012). These findings suggest that molybdenum has the potential to enhance plant growth, but its effects can vary depending on the plant species, formulation, and environmental conditions. Further research is needed to explore the full range of molybdenum's impact, especially under different stress conditions.

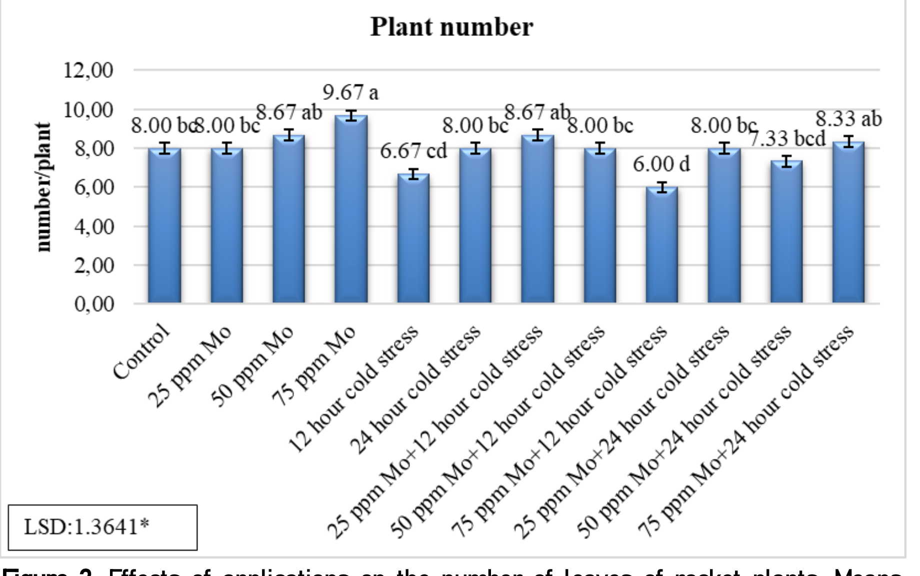

Figure 3. Effects of applications on the number of leaves of rocket plants. Means denoted by different lowercase in letters the same column indicate significant difference ($p<0.05$) among treatments

3.4. SPAD

The effects of the treatments on SPAD values were statistically significant (Figure 4; $p<0.001$). Significant differences in SPAD values were observed among the various concentrations of molybdenum (Mo) treatments compared to the control group (46.17 SPAD value). The 50 ppm Mo treatment exhibited the lowest SPAD value at 41.700, while the other Mo treatments recorded higher values, specifically 46.833 for 25 ppm Mo and 47.97 for 75 ppm Mo. Cold stress (both 12-h and 24-h) also had a notable impact on SPAD values. After 12 h of cold stress, the SPAD values were recorded as 39.13 and 38.13, while after 24 h of cold stress, the values were 38.767 and 38.53. It was observed that cold stress influenced the SPAD values of the Mo treatments during both time periods. Notably, after 12 h of cold stress, an increase in SPAD values was seen with Mo applications, such as 48.13 for 25 ppm Mo and 40.27 for 50 ppm Mo. However, under 24 h of cold stress, the effects of Mo treatments were more limited, with no significant increase in SPAD values (for example, 38.77 for 25 ppm Mo and 38.53 for 50 ppm Mo).

In conclusion, the impact of Mo treatments on SPAD values varied depending on the concentration of Mo and the duration of cold stress. The results of our study demonstrate that molybdenum (Mo) treatments significantly influence leaf chlorophyll content, as measured by SPAD values. Higher molybdenum concentrations (25 ppm and 75 ppm) generally enhanced SPAD values, with the 75 ppm Mo treatment showing the highest value (47.97), while the 50 ppm Mo treatment showed a lower value (41.70). This suggests that molybdenum positively affects chlorophyll content and, consequently, photosynthetic potential. These findings align with previous studies indicating that Mo enhances leaf photosynthesis, which can increase yield (Long et al., 2006; Ventura et al., 2010). Molybdenum plays a crucial role in enzymatic and biochemical processes that influence photosynthesis and stomatal functioning (Imran et al., 2019). For instance, Syaifudin et al. (2023) observed that nano-Mo fertilizer improved photosynthetic efficiency in winter wheat by increasing CO_2 concentration and transpiration rate, thereby enhancing carbon metabolism. In our study, cold stress significantly reduced SPAD values, reflecting a decrease in chlorophyll content and photosynthetic activity. However, the Mo treatments mitigated some of the cold stress effects, particularly after 12 h of exposure, with an increase in SPAD values in the 25 ppm Mo (48.13) and 50 ppm Mo (40.27) treatments. After 24 h of cold stress, the impact of Mo was less pronounced, suggesting that prolonged cold stress may limit the effectiveness of Mo in maintaining photosynthetic performance. This underscores the role of molybdenum in improving photosynthesis under stress conditions, although its effectiveness may depend on both the concentration of Mo and the duration of the stress.

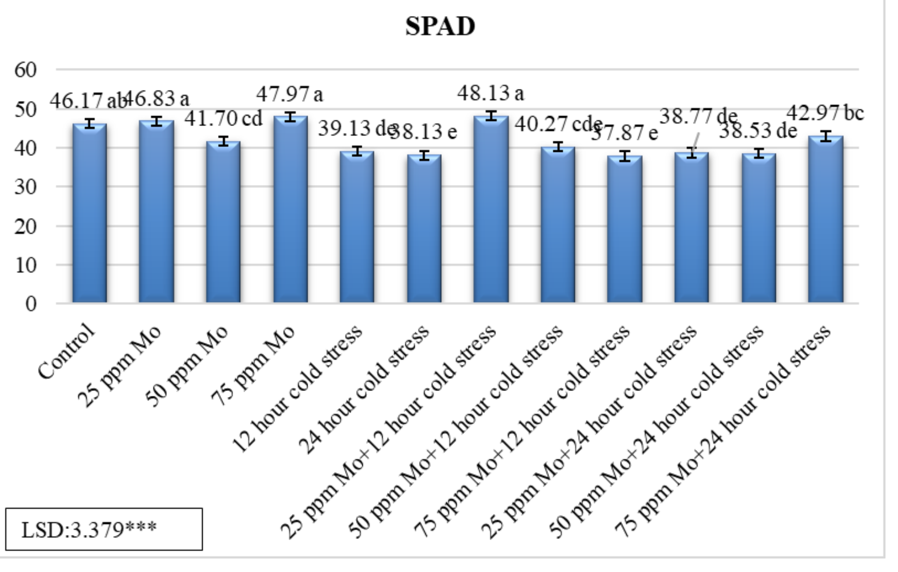

Figure 4. Effects of applications on SPAD value of rocket plant. Means denoted by different lowercase in letters the same column indicate significant difference (p<0.05) among treatments

3.5. Plant fresh weight

The effects of the treatments on plant fresh weight were found to be statistically significant (Figure 5; p<0.001). The control group exhibited the highest fresh weight value at 9.50 g. The effects of molybdenum (Mo) applications on plant fresh weight showed considerable variability. The 25 ppm Mo treatment resulted in a higher fresh weight (10.22 g) compared to the control group. Conversely, the 50 ppm Mo and 75 ppm Mo treatments had significantly lower fresh weight values at 4.88 g and 5.21 g, respectively. Cold stress (both 12-h and 24-h) also had a notable impact on plant fresh weight. After 12 h of cold stress, the fresh weight was measured at 8.02 g, while after 24 h of cold stress, it increased to 8.56 g. This indicates that cold stress has a detrimental effect on plant development. When examining the effects of Mo applications in conjunction with cold stress, distinct influences were observed following 12 h of cold stress with 25 ppm Mo (5.05 g), 50 ppm Mo (9.35 g), and 75 ppm Mo (5.37 g). Notably, the 50 ppm Mo application yielded a higher fresh weight compared to the other Mo levels.

However, after 24 h of cold stress, the effects of Mo treatments were more limited. For instance, the fresh weights for 25 ppm Mo (7.73 g), 50 ppm Mo (7.27 g), and 75 ppm Mo (6.06 g) showed slight improvements compared to the control group, but these improvements were not as pronounced as those observed after 12 h of cold stress. The impact of molybdenum applications on plant fresh weight varies significantly depending on Mo concentration and duration of cold stress. Lower Mo levels and prolonged cold stress can adversely affect fresh weight, whereas optimal Mo concentrations may promote plant development. While our study suggests that molybdenum may have a beneficial effect at certain concentrations, prolonged cold stress appears to diminish its positive impact. On the other hand, Vural and Müftüoğlu (2012) found that lower molybdenum concentrations did not significantly enhance fresh weight, further emphasizing the importance of finding the optimal concentration of Mo for each plant species and environmental condition.

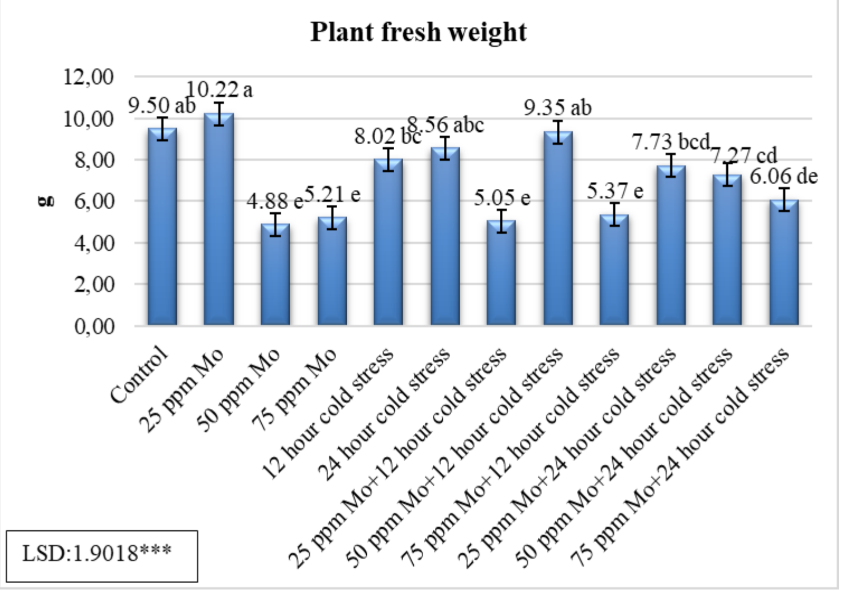

Figure 5. Effects of applications on plant fresh weight of rocket plant. Means denoted by different lowercase in letters the same column indicate significant difference (p<0.05) among treatments

3.6. Plant dry weight

The effects of the treatments on plant dry weight were not statistically significant (Figure 6; $p<0.001$). The control group exhibited a dry weight of 0.73 g. In assessing the impact of molybdenum (Mo) applications on plant dry weight, the 25 ppm Mo treatment resulted in a slightly lower dry weight of 0.72 g compared to the control group. The 50 ppm Mo and 75 ppm Mo treatments yielded dry weights of 0.73 g and 0.73 g, respectively, which were very close to the control group's value. The effects of cold stress (12 h and 24 h) on plant dry weight were evident, with the dry weight measuring 0.72 g after 12 h of cold stress and showing a significant reduction to 0.62 g after 24 h of cold stress. This indicates that prolonged cold stress adversely affects plant dry weight. When evaluating the effects of Mo applications in conjunction with cold stress, distinct influences were observed following 12 h of cold stress, with the dry weights for 25 ppm Mo (0.74 g), 50 ppm Mo (0.72 g), and 75 ppm Mo (0.61 g) demonstrating varied responses. Notably, the 25 ppm Mo treatment provided a slight increase compared to the other Mo levels. After 24 h of cold stress, the effects of Mo treatments became more pronounced. For instance, the dry weights for 25 ppm Mo (0.75 g), 50 ppm Mo (0.56 g), and 75 ppm Mo (0.56 g) exhibited differing effects compared to the control group. Both the 50 ppm Mo and 75 ppm Mo treatments resulted in significantly lower dry weight values. The impact of molybdenum applications on plant dry weight varies based on Mo concentration and duration of cold stress. Lower Mo levels and prolonged cold stress can significantly reduce plant dry weight, while optimal Mo concentrations may, in some cases, either increase or maintain dry weight at control group levels.

Comparing these findings to existing literature, Zou et al. (2008) suggested that appropriate Mo fertilization could increase wheat yields, which aligns with our observation that Mo application can influence plant growth, although the impact on dry weight was not as pronounced. Furthermore, Wen et al. (2019) reported an increase in dry matter yield in winter wheat when Mo was applied at 0.15 mg.kg−1, with a 14.27% increase over the untreated control. Our study did not show such a marked increase, but we did observe slight improvements with lower Mo concentrations (25 ppm Mo) under cold stress. Yu et al. (2002) also indicated positive effects of Mo application on dry matter yield in Mo-efficient wheat plants, which aligns with our findings that lower Mo concentrations could have a slight positive effect on dry weight, particularly in stressful conditions. Moreover, similar positive effects of Mo application on dry matter yield were reported in other crops, such as maize, rice, soybean, and oil rape seed (Kovács et al., 2015; Imran et al., 2020; Rana et al., 2020a, 2020b). While our results do not fully align with the more substantial increases reported in these studies, they do suggest that Mo's effectiveness is highly dependent on concentration and environmental stress factors, highlighting that lower concentrations may be more beneficial under specific stress conditions, such as cold stress, while higher concentrations may not always yield positive results.

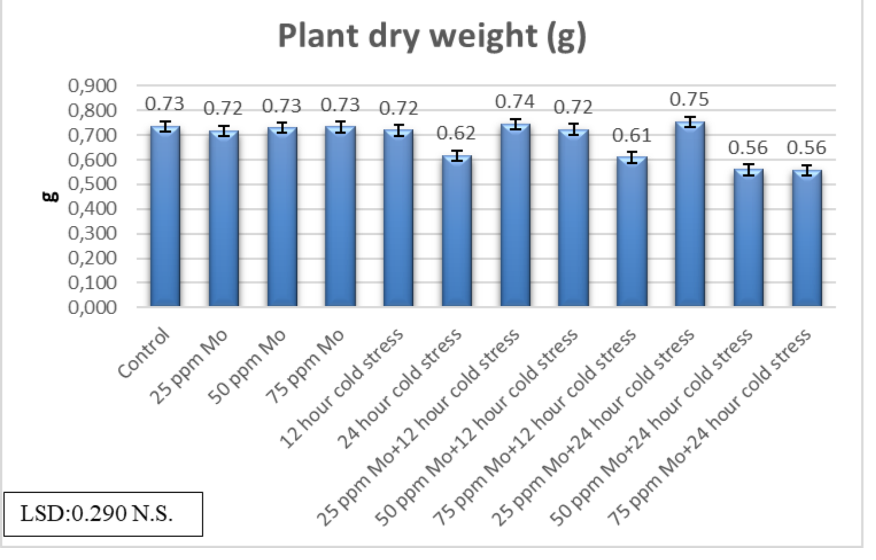

Figure 6. Effects of applications on plant dry weight of rocket plant. Means denoted by different lowercase in letters the same column indicate significant difference (p<0.05) among treatments

3.7. Plant moisture content

The effects of the treatments on plant moisture content were statistically significant (Figure 7; p<0.05). The control group exhibited a moisture content of 92.27%. In evaluating the impact of molybdenum (Mo) applications on plant moisture content, the 25 ppm Mo treatment resulted in a slightly higher moisture content of 93.00% compared to the control group. However, the 50 ppm Mo and 75 ppm Mo treatments displayed significantly lower moisture content values of 85.00% and 85.88%, respectively, when compared to the control group. The influence of cold stress (12 h and 24 h) on plant moisture content was also observed. After 12 h of cold stress, the moisture content was measured at 90.96%, while following 24 h of cold stress, the value increased to 92.51%. This indicates that cold stress has a significant effect on plant moisture content, with the 24-h duration producing a more pronounced impact. When examining the combined effects of cold stress and Mo applications, different influences were noted after 12 h of cold stress: the moisture contents for 25 ppm Mo (85.39%), 50 ppm Mo (92.20%), and 75 ppm Mo (88.56%) demonstrated varied responses. Notably, the 50 ppm Mo treatment provided a higher moisture content compared to the other Mo levels. After 24 h of cold stress, the effects of Mo applications varied: for instance, the moisture contents for 25 ppm Mo (89.36%), 50 ppm Mo (92.22%), and 75 ppm Mo (90.54%) produced different values relative to the control group.

The effects of molybdenum applications on plant moisture content significantly depend on Mo concentration and duration of cold stress. Lower Mo levels and prolonged cold stress can considerably decrease plant moisture content, while optimal Mo concentrations may, in some instances, increase moisture content or maintain it at control group levels. These findings are consistent with previous studies, such as one reporting that plant moisture values in broad bean plants ranged between 83.23% and 85.14%, with no significant statistical differences in moisture content between molybdenum treatments (Vural and Müftüoğlu, 2012). In contrast to our study, where Mo applications generally decreased moisture content at higher concentrations, their results showed that lower concentrations of Mo did not cause significant differences in moisture content. This discrepancy could be attributed to differences in plant species and experimental conditions, such as the duration and intensity of cold stress. While the highest moisture content in broad beans was found in plants without Mo, our study suggests that certain Mo concentrations, such as 50 ppm, may help plants retain moisture under specific stress conditions. These findings highlight the complexity of molybdenum's role in plant physiology, suggesting that its impact on moisture content is influenced by both the concentration of Mo and the duration of environmental stress. Further research is needed to determine the optimal Mo concentrations for maintaining moisture content across different plant species and environmental conditions.

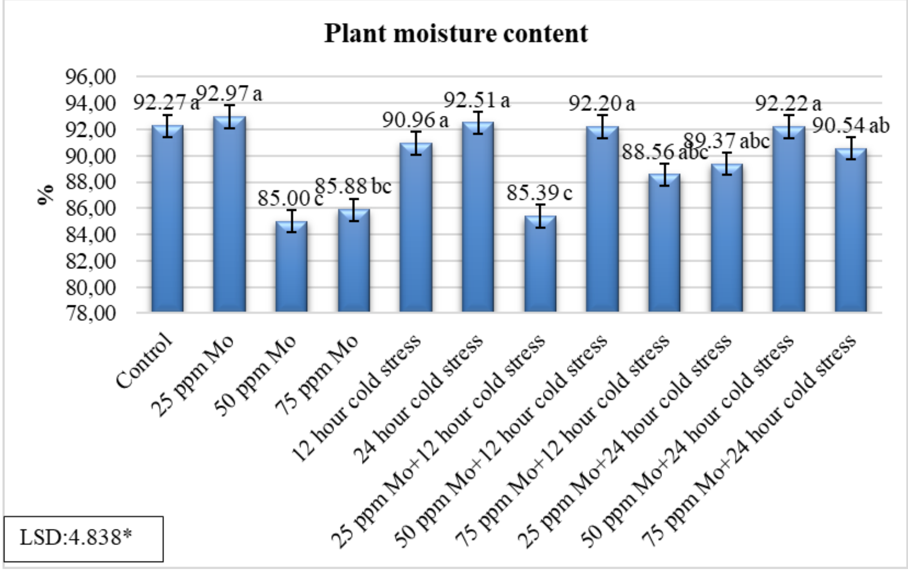

Figure 7. Effects of applications on plant moisture content of rocket plant. Means denoted by different lowercase in letters the same column indicate significant difference ($p<0.05$) among treatments

3.8. Relative water content (RWC)

The findings of this study indicate that the treatments applied in the cultivation significantly affected the Relative Water Content (RWC) of the plants (Figure 8; $p<0.001$). The control group represented a baseline RWC value of 90.21%. Molybdenum (Mo) applications resulted in notable variations in these values. Specifically, the 25 ppm Mo treatment exhibited a lower RWC of 84.34% compared to the control group, while the 50 ppm Mo treatment provided a value of 89.45%, which is nearly equivalent to that of the control. Conversely, the 75 ppm Mo application resulted in a significantly reduced RWC of 81.25%. Additionally, cold stress was found to have a significant effect on plant RWC values. For instance, after 12 h of cold stress, the RWC value was measured at 78.31%, whereas after 24 h, it increased to 83.781%. This indicates that prolonged cold stress may enhance plant moisture content. When evaluating the combined effects of cold stress and Mo applications, various outcomes were observed. For example, the combination of 12 h of cold stress with 25 ppm Mo resulted in a remarkably high RWC of 92.46%, demonstrating the potential of Mo to regulate moisture under cold stress conditions.

Conversely, when combined with 75 ppm Mo, the 12-h cold stress led to the lowest RWC value of 75.90%, suggesting that high concentrations of Mo may adversely affect moisture content. The effects of molybdenum applications on plant RWC values varied significantly depending on the Mo concentration and duration of cold stress. These findings contribute to our understanding of the complex effects of Mo on plant stress tolerance and water regulation. When comparing our results with those of Huang et al. (2023), who studied the effects of Mo and phosphorus on the RWC of two soybean varieties, Nakasennari and Sachiyutaka, some similarities and differences emerge. In the study on Nakasennari, the RWC under the LLMo0.2P100 treatment (0.2 mg Mo and 100 mg P kg^{-1}) was 84.7%, showing a 2.0% reduction compared to the control (LLCK), similar to our finding that Mo treatments at certain concentrations led to reduced RWC. Conversely, the Sachiyutaka variety exhibited a 1.3% higher RWC under the same treatment (LLMo0.2P100), suggesting that the effects of Mo on RWC may vary depending on the plant variety.

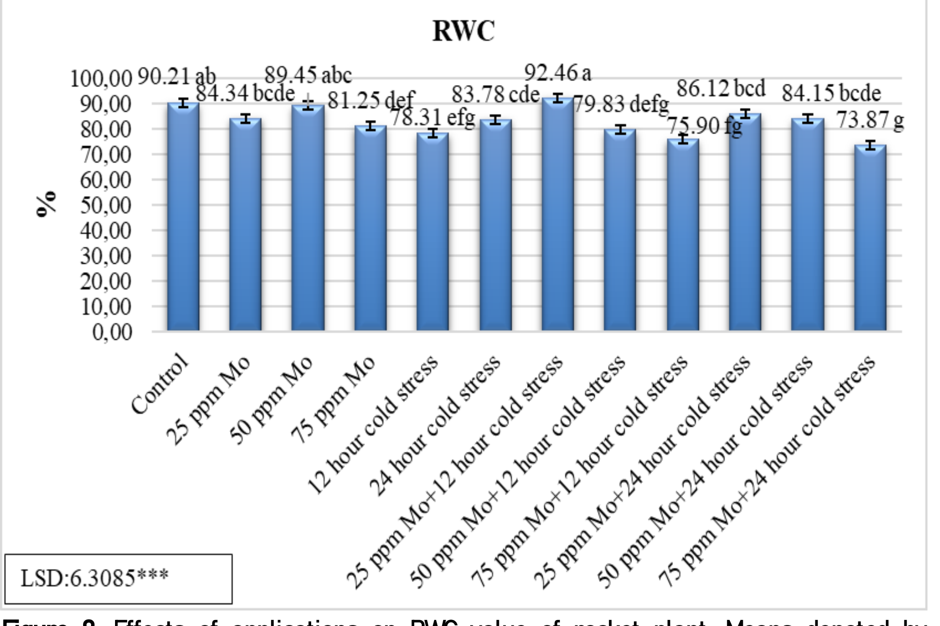

Figure 8. Effects of applications on RWC value of rocket plant. Means denoted by different lowercase in letters the same column indicate significant difference ($p<0.05$) among treatments

3.9. Turgor loss

The effects of the treatments on turgor loss were found to be statistically significant (Figure 9; $p<0.001$). This study investigated the impact of the treatments applied in the cultivation on plant turgor loss. The control group represented a baseline turgor loss value of 9.33%. Notable changes in this value were observed with molybdenum (Mo) applications. Specifically, the 25 ppm Mo treatment resulted in a significant increase in turgor loss, measuring 14.51%. The 50 ppm Mo application showed a value of 9.91%, which was similar to the control group, while the 75 ppm Mo treatment exhibited a higher turgor loss of 17.79%. The effects of cold stress (12 h and 24 h) on plant turgor loss were also evident. After 12 h of cold stress, the turgor loss was measured at 20.20%, indicating that cold stress increases turgor loss. However, after 24 h of cold stress, the turgor loss decreased to 15.41%, suggesting that prolonged exposure may have a mitigating effect. When assessing the combined effects of cold stress and Mo applications, various results emerged. For instance, the combination of 12 h of cold stress and 25 ppm Mo resulted in a significantly lower turgor loss of 7.04%. In contrast, the combination of 12 h of cold stress and 50 ppm Mo showed a turgor loss of 18.83%, which was higher than that of the control group. The highest turgor loss was observed with the combination of 12 h of cold stress and 75 ppm Mo, measuring 22.72%. Regarding the effects of Mo applications during 24 h of cold stress, the 25 ppm Mo treatment resulted in a turgor loss of 12.77%, which was close to the control group value. The 50 ppm Mo application resulted in a turgor loss of 14.55%, similar to that of the control.

However, the 75 ppm Mo application exhibited the highest turgor loss at 24.19%. These findings indicate that the effects of Mo on plant turgor loss significantly vary based on Mo concentration and duration of cold stress. Lower Mo levels and prolonged cold stress can increase turgor loss, whereas appropriate Mo concentrations may help maintain or reduce turgor loss to levels similar to those of the control group.

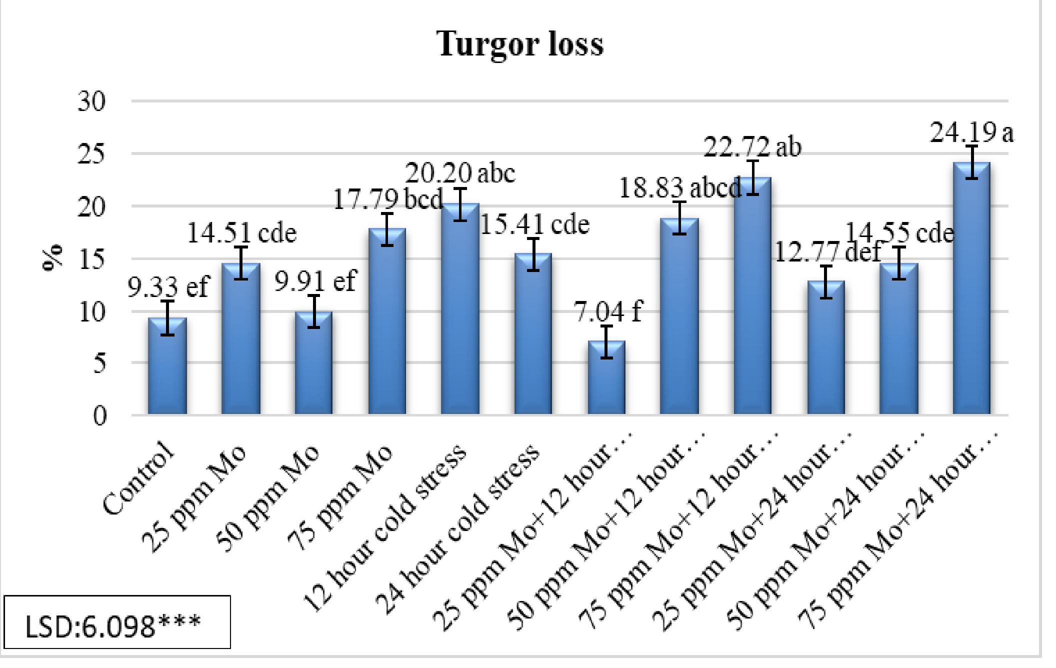

Figure 9. Effects of applications on turgor loss of rocket plant. Means denoted by different lowercase in letters the same column indicate significant difference (p<0.05) among treatments

3.10. Ion leakage

The effects of the treatments on ion leakage were found to be statistically significant (Figure 10; $p<0.05$). The control group represented a baseline ion leakage value of 13.32%. The molybdenum (Mo) applications showed varying effects on these values. Specifically, the 25 ppm Mo treatment resulted in an ion leakage of 13.18%, which was similar to the control group. In contrast, the 50 ppm Mo application showed a lower ion leakage value of 12.67%. The 75 ppm Mo application exhibited a slightly higher ion leakage value of 14.58% compared to the control group. Cold stress (12 h and 24 h) also had a significant impact on plant ion leakage. After 12 h of cold stress, the ion leakage was measured at 21.71%, indicating that cold stress increases ion leakage in plant cells. However, after 24 h of cold stress, the ion leakage decreased to 15.83%, suggesting a potential recovery or reduction in leakage over time. When evaluating the combined effects of cold stress and Mo applications, various outcomes were observed. For example, under 12 h of cold stress, the 25 ppm Mo treatment resulted in an ion leakage of 17.00%, which was slightly higher than the control group. The combination of 12 h of cold stress with the 50 ppm Mo application yielded an ion leakage value of 14.85%, which was similar to that of the control group. The 75 ppm Mo application combined with 12 h of cold stress resulted in an ion leakage of 14.54%, also close to the control. Assessing the effects of Mo applications under 24 h of cold stress revealed that the 25 ppm Mo treatment resulted in an ion leakage of 15.13%, again similar to the control group. The 50 ppm Mo application showed a lower ion leakage of 12.21% compared to the control, while the 75 ppm Mo application yielded an ion leakage of 13.27%, which was comparable to the control group. These findings indicate that the effects of Mo on ion leakage in plant cells vary significantly depending on the concentration of Mo and the duration of cold stress. It is important to note that lower Mo levels and prolonged cold stress can lead to increased ion leakage, whereas appropriate Mo concentrations may help maintain or reduce ion leakage to control levels.

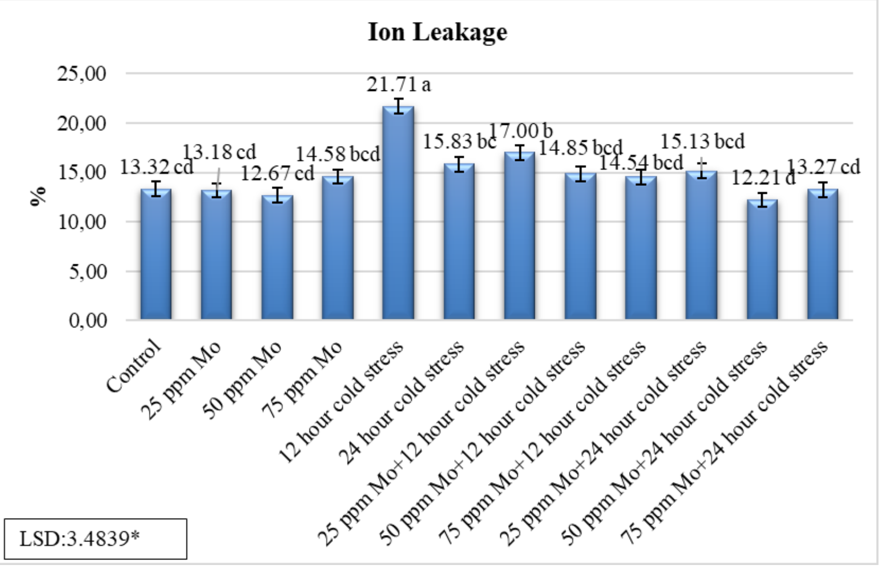

Figure 10. Effects of applications on ion leakage of rocket plant. Means denoted by different lowercase in letters the same column indicate significant difference (p<0.05) among treatments

4. Conclusion

The treatments had various effects on plant height, diameter, leaf number, SPAD value, fresh weight, dry weight, and moisture content. The different concentrations of Mo affected plant growth in distinct ways. Compared to the control group, the application of 25 ppm Mo resulted in a slight increase in plant height, while 50 ppm Mo showed a decrease, and 75 ppm Mo produced the highest increase. For plant diameter, a reduction was observed with 25 ppm Mo, whereas the 50 ppm Mo application was similar to the control group, and the 75 ppm Mo treatment resulted in an increase. In terms of leaf number, no change was noted with the 25 ppm Mo application, while a slight increase was observed with 50 ppm Mo, and a significant increase was recorded with 75 ppm Mo. Regarding SPAD values, the lowest measurement was found with 50 ppm Mo compared to the control, while the 25 ppm and 75 ppm Mo applications showed higher SPAD values. The fresh weight of the plants increased with the 25 ppm Mo application, while both 50 ppm and 75 ppm Mo applications significantly reduced fresh weight. Cold stress also adversely affected plant fresh weight. The Mo applications did not demonstrate significant effects on dry weight; however, cold stress considerably reduced dry weight. The 25 ppm Mo application increased plant moisture content, while the 50 ppm and 75 ppm Mo applications decreased it. Cold stress also had significant effects on moisture content.

In conclusion, molybdenum exhibited complex effects on plant growth, with these effects varying according to Mo concentration and the duration of cold stress. These findings provide an important foundation for the optimal use of molybdenum in agricultural practices and the development of strategies to protect plants against cold stress.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Sultan DERE: Investigation, Writing, Methodology, Investigation, Conceptualization, Validation, Original draft, Review and Editing. **Ali Yusuf KAYA**: Growing plants, Formal analysis, Data curation, Investigation, Writing.

Ethical approval

There is no need for an ethics committee report for the study.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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