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# Effect of molybdenum application in pepper (*Capsicum annuum* L.) under cold stress conditions

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**Abstract**

Cold stress (low temperature stress) is one of the abiotic stress factors. It causes many morphological and physiological problems in plants. One of the applications to eliminate and alleviate these negative effects is molybdenum application. The aim of this study was to determine the effect of molybdenum application on cold stress in commercial variety Mazamort pepper. In the experiment, control, 25 ppm molybdenum concentration, 72 hours cold stress and 25 ppm molybdenum +72 hours cold stress applications were included. Pots of 2 L were used to grow the plants and the growing medium was a mixture of peat and perlite in a ratio of 2:1 by volume. Climatic chamber conditions were set to 24±1°C during the day and 18±1°C at night with 16/8 h light/dark photoperiodicity for control conditions and 24±1°C during the day and 5±1°C at night with photoperiodicity for cold stress conditions. The experiment was planned according to the random plots factorial design with 3 replications and 6 plants in each replicate. At the end of the study, plant height, stem diameter, number of leaves, plant fresh and dry weight, SPAD, wet basis moisture content, leaf proportional water content and ion leakage parameters were analysed. The highest plant height of Mazamort pepper variety was determined in 25 ppm molybdenum+72 hours cold stress application (44.51 cm). Application of 25 ppm molybdenum was effective in alleviating the negative effect of cold stress on plant stem diameter, plant fresh-dry weight and turgor potential. Moisture content wet basis was lowest in 25 ppm molybdenum +72 hours cold stress application. SPAD value in pepper plants decreased under cold stress conditions. It was observed that 25 ppm molybdenum application was ineffective and the decrease increased under cold stress conditions. Ion leakage in Mazamort pepper variety was highest under 72 hours cold stress and 25 ppm molybdenum +72 hours cold stress conditions. Under cold stress conditions, 25 ppm molybdenum application was ineffective. Molybdenum application under cold stress conditions was found to have positive effects on some parameters in general. In future studies, we believe that the application of different molybdenum concentrations and different cold stress periods will reveal the effects of molybdenum more clearly.

**Keywords:** Pepper, Molybdenum, Cold stress, Low temperature, Abiotic stress

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## Introduction

Pepper (*Capsicum annuum* L.), a member of the Solanaceae family, is one of the most important vegetable crops grown worldwide, with economic value as a spice, medicine, vegetable and biopesticide (Lim et al., 2018b; Bea et al., 2021). It is important in terms of consumption after tomatoes and onions worldwide

(Ocharo et al., 2017). Although the demand for chillies is increasing worldwide, their productivity can be limited to varying degrees due to unfavourable environmental conditions such as dehydration, high salinity, low and high temperatures. To address this problem, numerous studies have focused on defence mechanisms activated in response to such environmental stresses (Chen et al., 2014; Guo et al., 2014; Park et al., 2016; Lim et al., 2018a, 2020; Kang et al., 2020; Wu et al., 2020). Pepper is a species native to tropical and subtropical regions. The optimum temperature required for germination and plant development is between 25°C and 30°C, and temperatures below 15°C are detrimental to germination and fruit set (Lorenz and Maynard, 1988). Chilling temperatures, which are often encountered in unheated greenhouses during autumn or winter production, can adversely affect fruit set of marketable fruits due to poor pollination and delay ripening and earliness in production (Sharaf-Eldin et al., 2022). In addition, the growth and development of pepper, especially in the reproductive stage, is affected by cold stress in early spring.

In many parts of the world, cold stress is one of the most important problems in agricultural production. Approximately 25% of the terrestrial area of the world consists of regions that do not drop below 15 °C and are safe from freeze damage, while the remaining 75% consists of regions where the temperature drops below 0 °C during certain periods. In these regions, sensitive plants can be damaged (Gözen and Kuşvuran, 2021; Aslantaş et al., 2010; Peşkircioğlu et al., 2016). Cold stress (low temperature stress) is one of the environmental stress factors that economically limit plant growth, crop productivity and quality, and post-harvest life. Cold stress occurs at temperatures above 0°C (usually 0-15°C). In low temperature stress, it causes damage to plant tissues without forming ice crystals. Most tropical and subtropical plants belong to this group. The damage to the plant is defined as chilling or cold damage (Gözen and Kuşvuran, 2021; Chen, 1994; Hasanuzzaman et al., 2013; Kumar et al., 2018). Symptoms of damage vary depending on factors such as the temperature and duration of exposure, genotype, developmental stage and light intensity of the environment (Gözen and Kuşvuran, 2021).

Each plant species has different optimum temperature limits. As a result of cold stress, cellular changes such as changes in the structure and composition of membranes, decreased protoplasmic flow, electrolyte leakage and plasmolysis occur in cold-sensitive plants. Depending on the severity of stress, metabolic changes such as increased or decreased respiration, production of unusual metabolites due to anaerobic state also occur (Kumar et al., 2018; Gözen and Kuşvuran, 2021). This situation shows that metabolic and physiological events are negatively affected and enzymatic activity decreases in sensitive plants under low temperature stress. Furthermore, osmolytes (such as amino acids, sugars and $K^+$) and products of photosynthesis leak out through the plasma membranes (Guy et al., 1992). Physiological damage to many plant tissues below 15°C and above the freezing point is called 'chilling injury'. Chilling damage is seen in plants as the formation of surface lesions, tissue water absorption, water loss, drying or shrinkage, internal discolouration, tissue degradation, accelerated senescence and ethylene production, changes in cell integrity due to leakage of plant metabolites, reduced growth, wilting and increased putrefaction (Lukatkin et al., 2012; Gözen and Kuşvuran 2021). In the study to identify lines resistant to low temperature stress in tomato genotypes, three pure lines tolerant to cold were identified in the results of electrolyte leakage and dry matter yield parameters (Tepe et al., 2022). It was reported that electrolyte leakage increased in tomato exposed to cold stress (4°C) for 3 days. Cold stress tolerant tomato lines have been reported to show low electrolyte leakage (Cao et al., 2015).

In recent years, different applications have been made to reduce the negative effects of cold stress. One of these applications is chemical applications. Molybdenum (Mo) is one of the chemicals used to reduce the negative effects of cold stress. Mo is a very important and essential micronutrient for plants, animals and bacteria (Rana et al., 2020a; Ismael et al., 2018). It has a major role within the plant system although only required in small amounts. Molybdenum application beneficial to increase plant growth (Müftüoğlu et al. 2021). Mo uptake is low in acidic media, so foliar Mo application was important (Bambara and Ndakidemi, 2010). Mo is one of the components of nitrate reductase and nitrogenase in nitrogen metabolism in plants (Zhang et al., 2012). The amount of molybdenum in the soil and its uptake by the plant directly affect symbiotic N fixation in legumes (Gök, 1993; Haktanır and Arcak, 1997; Durrant, 2001; Ferreira et al., 2002). Mo is utilized by certain plant enzymes in the process of reduction and oxidative reactions (Mendel and Hansch, 2002). An integral part of an organic pterin complex is called a molybdenum co-factor (Moco). Most higher plants have molybdoenzymes (enzymes that require molybdenum) and bind to Moco plants (Zimmer and Mendel, 1999; Kaiser et al., 2005; Mendel and Kruse, 2012; Bittner, 2014; Kovács et al., 2015). Mo is known to be involved in phosphorus and sulphur metabolism (Mendel and Hansch, 2002; Liu et al., 2010; Zhang et al., 2012). Mo also plays an important role in resistance to many abiotic stresses in plants. Winter wheat under cold stress has been shown to benefit from Mo application in terms of photosynthetic rates and products (Yaneva et al., 1996). When winter wheat was under drought stress, Mo application had a positive impact on photosynthetic rates and products (Zakhurul et al. 2000). By enhancing the activities of antioxidant enzymes, Mo also improved the cold tolerance of turf grasses (Yu et al., 2005). In the study conducted by Sun et al. (2009), it was reported that application of Mo increased the resistance of winter wheat to cold stress. It was reported that application of three amounts of Mo (0, 0.15, 0.3 mg $kg^{-1}$) to Chinese cabbage under salt stress significantly increased fresh weight; significantly improved the activities of antioxidant enzymes such as peroxidase, superoxide dismutase and catalase; significantly increased the content of non-enzymatic antioxidants such as glutathione, carotenoids and ascorbic acid. A significant increase in osmotic adjustment products such as soluble low molecular weight sugar, soluble protein and proline was also observed. In addition, Mo was reported to significantly increase the level of potassium ions ($K^+$) and improve the $K^+/Na^+$ ratio by decreasing the level of sodium ions (Na+). At the end of the study, Chinese cabbage was reported to improve its tolerance to salt stress by increasing its capacity to eliminate active oxygen and its osmotic adaptability (Zhang et al., 2014).

However, there is no report on whether Mo fertiliser application creates resistance to cold stress, especially in pepper plants. Therefore, the aim of our study was to reveal some physiological and morphological effects of Mo application in Mazamort pepper variety under cold stress conditions.

## MATERIALS AND METHODS

This study was carried out in the climate chamber and laboratory of Siirt University Faculty of Agriculture. Mazamort pepper variety was used as plant material and this variety was purchased from Sunagri Seed Company. This variety is a widely used commercial variety. Seeds are not hybrid. They are local vegetable seeds collected as a result of research from various regions of Anatolia. It is a variety which is cultivated under greenhouse and also suitable for open field cultivation. Fruits are 10-12 cm long, crisp, sweet, smooth shaped, dark green coloured and have three tips. It is used for edible. For the sowing of pepper seeds, peat and perlite were mixed in 2:1 ratio and sown. After the seeds were sown and irrigation was done. One month pepper seedlings (4-5 leaf stage) were transferred to plastic pots in a volume of 2 liters. The application was started 15 days after the pepper seedlings were transplanted. In the study, control, 25 ppm Mo concentration, 72 hours cold stress and 25 ppm Mo+72 hours cold stress applications were applied. Molybdenum is a brand of Alfa Aesar and was purchased from BigMed. Molybdenum concentration was determined by conducting preliminary studies. In our previous preliminary studies, cold stress was applied for 12 hours and 24 hours with 25 ppm, 50 ppm and 75 ppm molybdenum doses on different species and cultivars. The climate chamber conditions were set at an average humidity of 60–65% and a light intensity of 8000 lux. Pots of 2 L were used to grow the plants and the growing medium was a mixture of peat and perlite in a ratio of 2:1 by volume. Climatic chamber (19 $m^2$) conditions were set to 24±1°C during the day and 18±1°C at night with 16/8 h light/dark photoperiodicity for control conditions and 24±1°C during the day and 5±1°C at night with photoperiodicity for cold stress conditions. In pepper, the application was started before the flowering stage. Molybdenum application was applied as a spray every other day at the same time. Molybdenum application was performed 9 times. Control plants were sprayed with distilled water at application times.

The experiment was planned according to the random plots factorial design with 3 replications and 6 plants in each replicate. It was planned as 1 plant in each pot. Plants were irrigated with standard nutrient solution during the experiment. Sample pots were kept in order to determine the amount and time of irrigation in order to prevent the water holding capacity to be different in the pots. Pot plates were placed in the pots of each application and irrigation was made to reach the saturation point and the amount of irrigation was calculated by considering the amount of water drained. The ratio of "drained solution/applied solution" was taken as basis in irrigation (Schröder and Lieth, 2002). Drainage levels were determined and this ratio was adjusted to approximately 30-32% during the experiment. The pH and EC of the drained water were measured irrigation at times.

Nutrient content was prepared using Hoagland nutrient solution. The pH of the nutrient solution was kept between 6.0-6.5 and EC between 1.5-2.5 dS $m^{-1}$. Plant measurements were made 54 days after sowing. Plant height, stem diameter, number of leaves, plant fresh and dry weight, SPAD measurement, wet base moisture content, leaf proportional water content and ion leakage parameters were analysed in at least 4 plants.

### Determination of Plant Height

At the end of the experiment, the plant was measured from the root collar to the growing tip with a metre and recorded in centimetre (cm).

### Determination of Plant Stem Diameter

Plant stem diameter was measured using compass and recorded in millimetre (mm). Plant stem diameter were made 54 days after sowing.

### Determination of the Number of Leaves

All leaves on the plant were counted at the end of the study and the number of leaves was determined as number/plant.

### Plant Fresh Weight

All green parts were weighed on a precision balance and recorded in grams (g).

### Plant Dry Weight

After the plant fresh weights were taken, the plant samples were dried in an oven at 75°C for 48 hours and recorded in g (Arshadullar and Zaidi, 2007).

### Measurement with SPAD Meter for Chlorophyll

Readings were taken with a Minolta SPAD meter to determine the tone of green in young, middle-aged and young leaves of pepper plants depending on the amount of chlorophyll (Daşgan et al., 2010).

### Wet Basis Moisture Content (%)

Wet basis moisture content was determined by using fresh weight and dry weight of the plants according to the following formula (Koksal et al., 2016).

$$MCwb = ((FW - DW)/FW)*100$$

MCwb: Moisture content wet basis (%), FW: Plant fresh weight (g), DW: Plant dry weight (g)

### Leaf Proportional Water Content (RWC)

Five pieces of 1 cm discs were taken from fresh plant leaves and weighed and recorded. After the leaves were kept in pure water for 24 hours, the leaves were removed from the water, dried and their turgor weights were determined. The leaves whose turgor weights were determined were kept in an oven at 70°C for 24 hours. After drying, the dry weight was taken in grams. Leaf proportional water content (%) was calculated by placing the values in the following formula (Van Laere et al., 2011).

$$RWC = (FW-DW)/(TW-DW)*100$$

FW: Fresh Weight DR: Dry Weight TW: Turgor Weight

### Ion Leakage

The 3rd leaves of the plant from the growth tip were used for this purpose. For this purpose, 1 cm diameter leaf discs were kept in de-ionised water for 5 hours and then EC was measured (EC1), the same discs were kept at 75°C for 24 hours and then the EC value of the solution (EC2) was measured again. Ion leakage was calculated as % using the formula (Arora et al., 1998).

$$\text{Ion leakage} = (EC1/ EC2)*100$$

### Statistical Analysis

The significance between control, molybdenum application, cold stress and molybdenum+cold stress application was evaluated by analysis of variance (ANOVA) test. In case ANOVA showed significant differences between control, molybdenum application, cold stress and molybdenum+cold stress application, Least Significant Difference (LSD) test ($P \leq 0.05$) was used to compare the means. Differences in the data were evaluated using JMP 8th statistical software (Steel et al., 1997).

## RESULTS AND DISCUSSION

Molybdenum is a rare element that is essential for plant growth and can be obtained from soil (Kaiser et al., 2005). However, at high concentrations Mo is known to have a negative effect on plant growth (Rihan et al., 2014). Many studies have reported that Mo application may have ameliorative effect against frost (Du et al., 1994; Li et al., 2001) and cold stress (Sun et al., 2006; Al-Issawi et al., 2013) damage.

The difference among applications in terms of plant height was found statistically significant ($p \leq 0.05$). Plant height values are shown in Figure 1. Among the applications, the highest plant height was determined in 25 ppm Mo+72 hours cold stress application with 44.51 cm and the lowest plant height was determined in 72 hours cold stress with 38,75 cm. It was determined that plant height increased in 25 ppm Mo application under 72 hours cold stress conditions. Plant height was higher in 25 ppm Mo application and 25 ppm Mo+72 h cold stress application compared to control. The highest plant height of Mazamort pepper variety was determined in 25 ppm Mo+72 hours cold stress application (44.51 cm). Molybdenum application was effective in alleviating the negative effect of cold stress on plant height. Molybdenum application under control conditions positively affected plant height. It has been reported that prolonged cold stress conditions cause a decrease in plant height (Hassan et al., 2021).

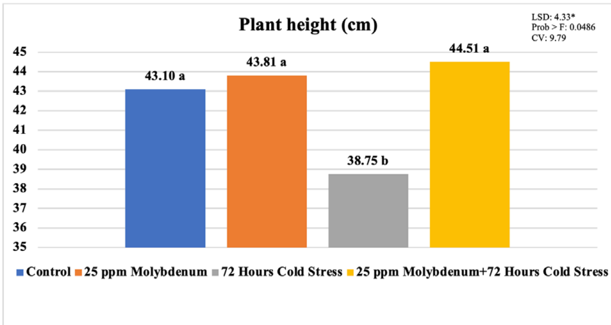


**Figure 1.** Effect of applications on plant height

The values of the applications in terms of plant diameter are shown in Figure 2. Plant diameter values of the applications were not statistically significant ($p \leq 0.05$). The highest plant diameter among the applications was in the control application. The lowest plant diameter was 6.14 mm in 72 hours cold stress application. Plant diameter was 6.98 mm in 25 ppm Mo application under 72 hours cold stress. Plant diameter decreased in other applications compared to the control, but the least decrease was determined in 25 ppm Mo+72 hours cold stress application. Cold stress decreased the plant diameter. The negative effect of cold stress was alleviated by 25 ppm Mo application under cold stress conditions.

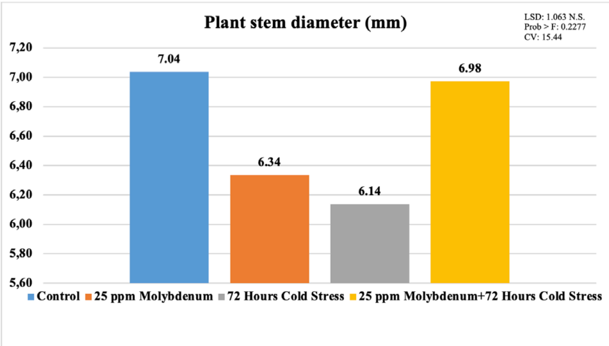


**Figure 2.** Effect of applications on plant diameter

The number of leaves was found to be statistically insignificant ($p \leq 0.05$). The number of plant leaves is shown in Figure 3. The number of leaves varied between 25.75 and 29.75 number/plant. The highest number of leaves (29.75 number/plant) was obtained in the control application. The lowest number of leaves was in Mo application with 25.75 number /plant. While the number of leaves was 27.25 number/plant under cold stress, it was 27.00 number/plant in 25 ppm Mo application under cold stress. The number of leaves of Mazamort pepper variety differed among applications. The lowest number of leaves was in Mo application compared to the control. It was determined that cold stress decreased the number of leaves and 25 ppm Mo application did not stop this decrease. It was determined that 30 ppm application of Mo decreased micro shoot growth. It was also reported that Mo application increased the average plant weight under low temperature stress (Rihan et al., 2014).

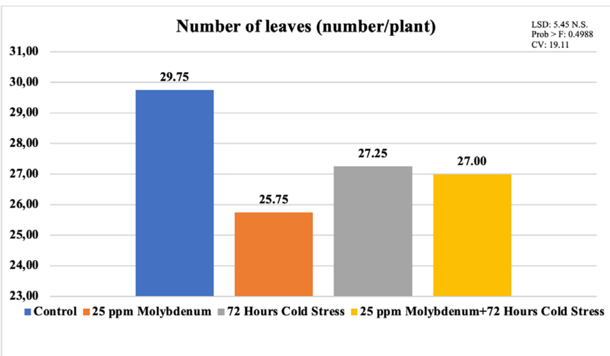


**Figure 3.** Effect of applications on the number of leaves

It was found that plant fresh weight of Mazamort pepper variety differed between applications, but this difference was not statistically significant ($p \leq 0.05$). The plant fresh weight obtained as a result of the applications was shown in Figure 4. Plant fresh weight varied between 22.78 g and 27.48 g. The highest plant fresh weight was in the control application (27.48 g) and the lowest was in the 72 hours cold stress application (22.78 g). Higher plant fresh weight was obtained in Mo application than 72 hours cold stress and 25 ppm Mo+72 hours cold stress application. Plant fresh weight decreased under cold stress conditions. Application of 25 ppm Mo was effective in alleviating the negative effect of cold stress on plant fresh weight. It has been reported that leaf size, leaf area and shoot biomass are reduced under cold stress (Valluru et al., 2012). It was reported that the combined application of cold and freezing stress caused chlorosis and a decrease in shoot biomass compared to the control (Hassan et al., 2021). It was reported that Mo application increased the micro shoot weight (Rihan et al., 2014).

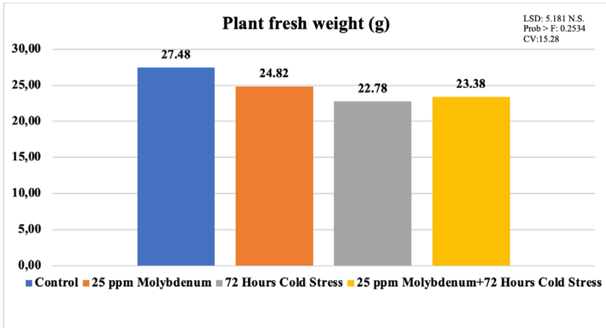


**Figure 4.** Effect of applications on plant fresh weight

It was determined that the applications had no statistically significant effect on plant dry weight ($p \leq 0.05$). Plant dry weight obtained as a result of the applications is shown in Figure 5. The highest plant dry weight was 2.57 g in the control application and the lowest was 2.22 g in the 72 hours cold stress application. While the plant dry weight was 2.22 under cold stress conditions, 25 ppm Mo+72 hours cold stress application increased the plant dry weight to 243 g. The lowest plant dry weight of Mazamort pepper variety was found under cold stress. It was found that 25 ppm Mo application was effective under cold stress conditions and increased plant dry weight under cold stress. Molybdenum application also had a negative effect on plant dry weight in control conditions. Mo application was reported to increase plant dry weight (Imran et al., 2019).

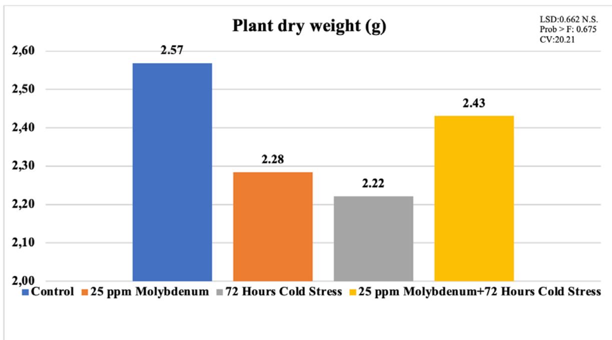


**Figure 5.** Effect of applications on plant dry weight

Pepper moisture content wet basis was shown in Figure 6. The moisture content of Mazamort pepper variety differed among the applications, but it was not statistically significant ($p \leq 0.05$). The highest moisture content was in 25 ppm Mo application with 90.81% and the lowest was in 25 ppm Mo+72 hours cold stress application with 89.53%. Moisture content wet basis was lowest in 25 ppm+72 hours cold stress application. It was observed that cold stress decreased the moisture content wet basis and 25 ppm Mo application was ineffective in alleviating this decrease. Under control conditions, Mo application increased the moisture content wet basis.

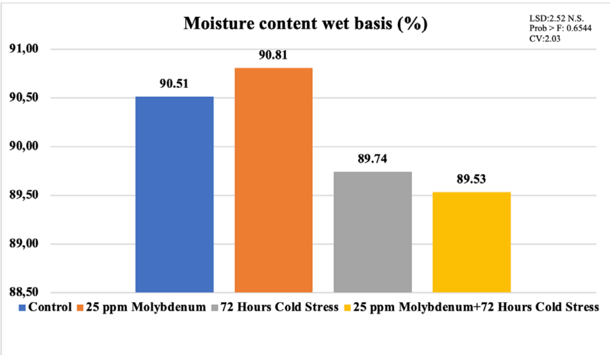


**Figure 6.** Effect of applications on moisture content of wet basis

SPAD value of Mazamort pepper variety showed a difference between applications and this difference was found statistically significant ($p \leq 0.0001$). SPAD value is shown in Figure 7. The highest SPAD value was in control application with 34.78 and the lowest was in 72 hours cold stress+25 ppm Mo application with 29.46. SPAD value decreased in other applications compared to control. SPAD value, which is important in determining the amount of chlorophyll in the plant, was the highest under control conditions. SPAD value in pepper plants decreased under cold stress conditions. It was observed that 25 ppm Mo application was ineffective and the decrease increased under cold stress conditions. The main damage site of cold stress is the chloroplast and photosynthesis. Tolerance in these aspects is expressed in native vegetation adapted to cold conditions (Sanghera et al., 2011). Yield losses occurring under cold stress conditions have been reported to be associated with low leaf area and reduced photosynthetic capacity. It has been stated that prolonged cold stress conditions cause leaf chlorosis (Hassan et al., 2021). Cold stress conditions affect cellular function due to changes in the photosynthetic apparatus (Manasa et al., 2022).

The difference in turgor potential of Mazamort pepper variety between applications was found statistically significant ($p \leq 0.0001$). RWC value is shown in Figure 8. RWC value varied between 93.80% and 96.31%. We think that some chemical applications can be effective in increasing plant growth and content, therefore, 25 ppm Mo application increased RWC. RWC decreased in 72 h cold stress and 25 ppm Mo+72 h cold stress application compared to the control. While RWC was 93.80% in cold stress, RWC increased to 94.36% in 25 ppm Mo application under cold stress conditions. Under control conditions, 25 ppm Mo application increased RWC. Water and nutrient relations have been stated to deteriorate in plants exposed to prolonged cold stress conditions (Hassan et al., 2021). The turgor potential was the lowest in 72 hours cold application. In alleviating the negative effect of cold stress on turgor potential, 25 ppm Mo application was effective under cold stress conditions. In control conditions, Mo application had a positive effect on turgor potential and increased it. It was reported that root length was more sensitive to cold stress conditions than dry weight. It has been reported that root length decreases under cold stress conditions and this disrupts the balance of water and nutrient uptake (Hussain et al., 2018).

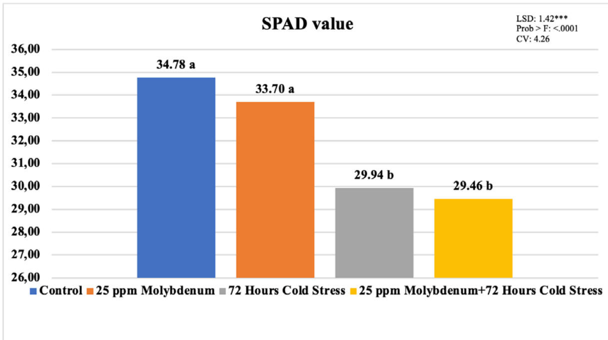


**Figure 7.** Effect of applications on SPAD value

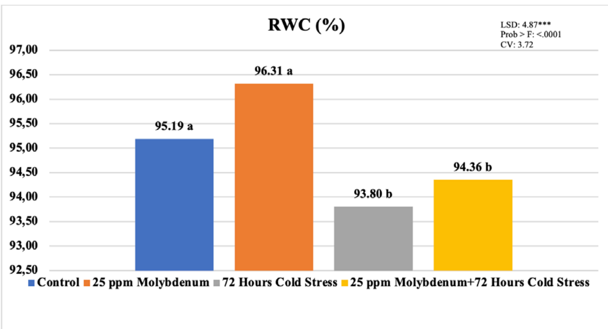


**Figure 8.** Effect of applications on RWC value

The difference among applications in terms of ion leakage was found to be statistically insignificant ($p \leq 0.05$). The ion leakage of Mazamort pepper variety as a result of the applications was shown in Figure 9. Ion leakage increased in other applications compared to the control. It was determined that 25 ppm Mo application did not reduce ion leakage under cold stress conditions and had the same rate of ion leakage with cold stress. The lowest ion leakage was in the control application with 17.32% and the highest was in the 72 hours cold stress and 25 ppm Mo+72 hours cold stress application with 17.88%. Changes in membrane fluidity occur during temperature stresses. This is a consequence of temperature stress damage and represents a potential sensing and/or damage zone (Horvath et al., 1998; Orvar et al., 2000). Ion leakage is an important parameter in determining the effect of stress in stress applications. Ion leakage in Mazamort pepper variety was highest under 72 hours cold stress and 25 ppm Mo +72 hours cold stress conditions. Under cold stress conditions, 25 ppm Mo application was ineffective. It has been shown that the primary site of freezing damage in plants is the membrane systems of the cell (Steponkus, 1984; Levitt, 1980). It is well known that freeze-induced membrane damage is initially caused by severe dehydration related with freezing (Steponkus, 1984; Steponkus et al., 1993). At non-freezing low temperatures, many species of tropical or subtropical origin are known to be damaged or killed. Various symptoms of chilling damage such as chlorosis, necrosis or growth retardation have also been reported. However, it has been reported that cold stress tolerant species continue to grow under cold conditions. Therefore, it is important to stabilise membranes in tolerance to cold stress (Sanghera et al., 2011). Under cold stress conditions, it affects cellular function due to changes in electron flow (Manasa et al., 2022). It was reported that Mo application was effective in reducing the negative effect of cold stress on ion leakage under cold stress conditions (Rihan et al., 2014).

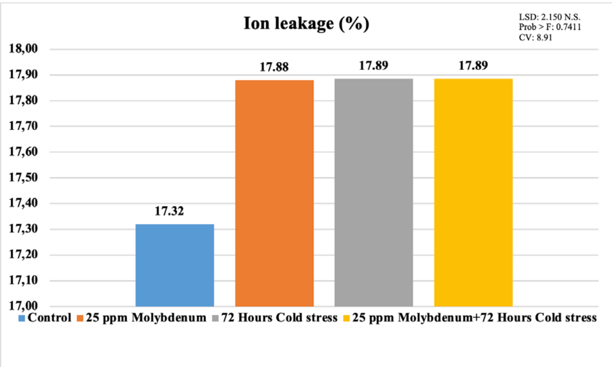


**Figure 9.** Effect of applications on ion leakage

## CONCLUSION

The highest plant height of Mazamort pepper variety was determined in 25 ppm Mo+72 hours cold stress application. Application of 25 ppm Mo was effective in alleviating the negative effect of cold stress on plant stem diameter, plant fresh-dry weight and turgor potential. Moisture content wet basis was lowest in 25 ppm Mo+72 hours cold stress application. SPAD value in pepper plants decreased under cold stress conditions. It was observed that 25 ppm Mo application was ineffective and the decrease increased under cold stress conditions. Ion leakage in Mazamort pepper variety was highest under 72 hours cold stress and 25 ppm+72 hours cold stress conditions. Under cold stress conditions, 25 ppm Mo application was ineffective. Mo application under cold stress conditions was found to have positive effects on some parameters in general. We believe that the application of different Mo concentrations and different cold stress periods in future studies will reveal the effects of Mo more clearly.

## COMPLIANCE WITH ETHICAL STANDARDS

This research article complies with research and publishing ethics.

**Peer-review**

Externally peer-reviewed.

**Conflict of interest**

The author of the article declares that there is no conflict of interest since he/she is the sole author

**Ethical committee approval**

Ethics committee approval is not required.

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It is a work of the author's own budget.

**Data availability**

Not applicable

**Consent for publication**

Not applicable.

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